THE ACTION OF ETHYLHYDROCUPREIN (OPTOCHIN) ON TYPE STRAINS OF PNEUMOCOCCI IN VITRO AND IN VIVO, AND ON SOME OTHER MICRO-ORGANISMS IN VITRO.

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(Received for publication, May 25, 1915.)

In 1911 Morgenroth and Levy (1) introduced the drug ethylhydrocuprein (optochin), a derivative of hydroquinine, in the treatment of experimental pneumococcic infection. Subsequent tests have, as a rule, shown the compound to possess a parasiticidal effect on the pneumococci. In the experiments so far reported, different strains of pneumococci, chosen more or less at hazard, were studied; no attempt, however, has been made to investigate the action of the drug on type strains of the various groups of pneumococci. It seemed of interest to investigate the action of the drug from this viewpoint.

Neufeld (2) showed that strains of pneumococci differed among themselves in respect to their immunity reactions (protective bodies). The investigations of Cole (3) and of Dochez and Gillespie (4) have resulted in a serological classification of the pneumococci. It has been shown by these authors that the pneumococci can be divided into at least four groups. The organisms of Groups I and II are specific in their immunity reactions; that is, an immune serum produced against any member of Group I has a specific protective action against, and a specific agglutinative action on, any member of Group I, but has no effect on any members of Groups II, III, or IV. Similarly, a serum immune to any member of Group II will react in the same way with all members of that group, but not with any members of Groups I, III, or IV. In Group III are included all members of the *Pneumococcus mucosus* type. To Group IV belong all pneumococci not belonging to Groups I, II, or III. A serum immune to any member of Group IV exhibits protective and agglutinative reactions with the strain used for its production, but in no instance with any other member of Group IV or

with any member of the other three groups. Lister (5) has arrived at a somewhat similar classification from opsonic studies.

EXPERIMENTAL.

The pneumococci used in the present study were representative of these four serological groups and were obtained from the Hospital of The Rockefeller Institute, with the exception of the microorganism designated as South Africa (9), which was obtained from the South African Institute for Medical Research; this organism has been placed in a fifth group by Lister (5) as a result of his opsonic studies. All strains of pneumococci used in the present study were isolated from cases of human pneumonia, except those called I Les and II White, which were obtained from cases of pneumococcal meningitis by lumbar puncture.

The Action of Ethylhydrocuprein in Vitro.

Wright (6) has shown that the action of ethylhydrocuprein hydrochloride on pneumococci *in vitro* is only slightly lessened by the presence of serum, while with antiseptics such as lysol and cresol, under similar conditions, the contrary is the case. Tugendreich and Russo (7) have shown that the hydrochloride of the drug in a concentration of 1 in 16,000 kills the pneumococcus *in vitro* in three hours at room temperature, and that it is much more potent in this respect than the hydrochlorides of the homologous compounds: isopropyl, isobutyl, and isoamylhydrocuprein; this superiority is even more marked over quinine and hydroquinine. In the present study the action of ethylhydrocuprein hydrochloride on type strains of the different groups of pneumococci was worked out and compared with that on other microorganisms under similar conditions.

Technique.—A solution of the drug was made in broth and sterilized by boiling or filtration through a Berkefeld filter. Various dilutions of this stock solution were made; to 2 cc. of each dilution in a test-tube 0.1 cc. of a 24 hour broth culture was added and the tubes were incubated for 18 hours at 37° C. At the end of this period the tubes were examined, and the results, as regards growth or inhibition of growth, noted; a large loopful of the two

TABLE I.

The Action in Vitro of Ethylhydrocuprein Hydrochloride on Some Representatives of the Four Serological Groups of Pneumococci.

Serological group of pneumococcus.	I.			II.			III.			IV.		
Designation of strain of pneumococcus....	106^5	$(B42)1^4$	Les	$(B21)1^{10}$	38^3	White	$(A66)14^2$	$(E22)4^3$	$(B48)1^4$	$(M)4^3$	$(A67)11^3$	South Africa (9)
Highest dilution causing inhibition of growth.....	500,000	1,000,000	500,000	1,000,000	1,000,000	1,000,000	500,000	10,000,000	1,000,000	500,000	1,000,000	1,000,000
Highest dilution causing death.....	20,000	1,000,000	100,000	1,000,000	50,000	1,000,000	100,000	10,000,000	100,000	50,000	1,000,000	1,000,000

lowest dilutions showing growth macroscopically, and of all tubes showing no growth, was in each case plated in about 12 cc. of blood agar and incubated. The concentration of the drug was thus enormously diluted. The plates were examined at the end of 24 hours and again at the end of 48 hours. Thus, one could differentiate between an inhibitory effect of the drug on the growth of the microorganism and actual death. Some typical results are given in Table I.

Explanation of Tables.—In the top row the Roman numeral stands for the serological group to which the strain of pneumococcus belongs; a letter and number in brackets, if present, denote the particular strain; the Arabic number represents the number of animal passages of the strain and the exponent the number of cultivations on artificial medium since the last animal passage. In the remaining two rows each number represents the greatest dilution in which the growth of the corresponding microorganism was inhibited or killed, as the case may be. In the case of the pneumococci the range of dilutions of the drug examined was as follows: 1 in 10,000; 1 in 20,000; 1 in 50,000; 1 in 100,000; 1 in 500,000; 1 in 1,000,000; and 1 in 10,000,000.

From the results given in Table I it is seen that ethylhydrocuprein hydrochloride causes in very high dilutions, *in vitro,* an inhibition of growth, and death of the pneumococcus, the latter occurring, generally speaking, in somewhat lower dilutions; and that no constant or considerable differences are seen in these effects on typical representatives of the four groups of pneumococci.

It was thought of interest to compare the action on pneumococci with that on other organisms, more especially streptococci. The results are set forth in Table II.

Description of the Microorganisms Mentioned in Table II.

Streptococcus 1 was a non-hemolytic streptococcus cultivated by Dr. Beattie from a case of rheumatic fever. The culture used was obtained from the Bacteriological Department of the Museum of Natural History, New York.

Streptococci 5 and 7 were two different strains of non-hemolytic streptococci obtained from the Pathological Department of Mt. Sinai Hospital, New York. They were cultivated from the blood of patients suffering from sabacute endocarditis.

TABLE II.

The Action in Vitro of Ethylhydrocuprein Hydrochloride on Microorganisms Other than Pneumococci.

Micro-organism.	*Micrococcus catarrhalis.*	*Streptococcus mucosus.*	Streptococcus 1.	Streptococcus 5.	Streptococcus 7.	Streptococcus 8.	Streptococcus 59.	Streptococcus R.	Streptococcus C.	*B. coli communis.*	*B. typhosus.*	*B. paratyphosus* B.	*Staphylococcus albus.*	B. Friedländer.
Highest dilution causing inhibition of growth	10,000	20,000	Not inhibited in 10,000	20,000	10,000	10,000	10,000	20,000	10,000	Not inhibited in 10,000	Not inhibited in 10,000	Not inhibited in 10,000	Not inhibited in 10,000	10,000
Highest dilution causing death	Not killed in 10,000	Not killed in 10,000	Not killed in 10,000	20,000	Not killed in 10,000	Not killed in 10,000	Not killed in 10,000	Not killed in 10,000	Not killed in 10,000	—	—	—	—	Not killed in 10,000

TABLE III.*

The Action in Vitro of Quinine Hydrochloride.

Microorganism.	I105[6].	II3[85].	IIIA66.12[4].	IVM.4[3].	*Streptococcus mucosus.*	*Micrococcus catarrhalis.*	Streptococcus I.	*B. coli communis.*	*Staphylococcus albus.*
Highest dilution causing inhibition of growth	50,000	50,000	50,000	50,000	5,000	5,000	5,000	1,000	1,000
Highest dilution causing death	5,000	10,000	5,000	10,000	1,000	5,000	2,000	1,000	1,000

* The first four organisms are representatives of the four groups of pneumococci.

Streptococcus 8 was a laboratory strain of hemolytic streptococcus.

Streptococcus 59 was a green-producing, non-hemolytic streptococcus cultivated by Dr. H. F. Swift from the blood of a patient suffering from rheumatic pericarditis.

None of these strains had a capsule, fermented inulin, or was bile-soluble.

The *Streptococcus mucosus* was a diplococcus growing in short chains; it showed, with His's stain, a well defined capsule in the body fluids of an infected animal; it was not bile-soluble; it was pathogenic for mice, causing a sticky exudate in the peritoneal cavity; it did not ferment inulin; on cultivation for a few passages on blood agar the growth, which was previously moist on solid media, became dry in character.

The streptococcus strains called R and C were isolated from normal sputum and were mucous in type; that is, they gave a mucoid growth on blood agar and possessed a capsule; these qualities were, however, lost after a few passages on artificial media. Neither was bile-soluble, and neither fermented inulin.

From Table II it is seen that the action, if any, of ethylhydrocuprein, in the dilutions examined, on the microorganism mentioned therein was considerably less than that on the pneumococci.

The action *in vitro* of the hydrochloride of quinine (from which ethylhydrocuprein is derived) on pneumococci and on some other microorganisms is shown in Table III. It is seen therefrom that, while the action of quinine hydrochloride is greater on the pneumococci than on the other bacteria, this action is far less in the former case than that of optochin.

The Action of Ethylhydrocuprein (Optochin Base) on Representatives of the Four Groups of Pneumococci in Vivo.

Owing to the fact that certain biochemical relationships (such as bile solubility) exist between trypanosomes (8) and spirilla (9), on the one hand, and the pneumococci (10) alone among the *Cocaceæ*, on the other, Morgenroth (1) investigated the action on the pneumococci of that group of compounds which has given results in the therapy of trypanosomal and spirillar infections; namely, quinine and its derivatives.

Morgenroth and Levy showed that ethylhydrocuprein (a derivative of hydroquinine) exerted a considerable protective action, and a certain degree of curative action (1) on experimental pneumococcal infection in the mouse. Later Gutmann (11) and Morgenroth and Kaufmann (12) reported experiments in which this protective action on several strains of pneumococci was shown. Levy (13) studied the curative effect of the drug on pneumococcal infection of the mouse by a strain of *Pneumococcus mucosus*, an interval of from two to six hours having been allowed to elapse between the intraperitoneal infection and the first

administration of the drug. The dosage of the infection does not seem to have been accurately measured in Levy's experiments, nor do the results seem to have been checked by autopsy; an effect of the drug, however, on the infection can be seen; of 16 mice, 10 survived up to the 8th day, when the observation was discontinued. This work is summarized by Rosenthal (14).

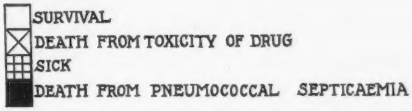
So far, the strains studied in this connection were chosen more or less at haphazard, no attempt having been made to study strains of pneumococci in relation to classification. In the following experiments typical representatives of the four groups of pneumococci were used.

Technique.—A 2 per cent solution of the free base ethylhydrocuprein (optochin base) was made in sterile olive oil by rubbing up in a mortar and standing the product so obtained in the incubator at 37° C. over night. Mice of 18 grams' weight and upwards were used. The infection was given in a constant volume of 1 cc. of broth intraperitoneally. The treatment consisted of 0.5 cc. of the 2 per cent optochin base solution in oil (calculated in every case) per 20 grams of mouse given under the skin of the back immediately after the infection; this was followed by an equal dose on the second day of the experiment, and by 0.4 cc. per 20 grams of mouse on the third and fourth days. The mice were observed for a period of from 10 to 14 days. An autopsy was performed on every mouse that succumbed (whether a treated animal or a control), and the heart's blood, and peritoneal fluid if necessary, were examined in a smear preparation, Gram's stain and His's capsule stain being used. If no Gram-positive and capsule-bearing bacteria were found in the smear by these methods, cultures were made on defibrinated rabbit blood agar with abundant inoculation, incubated for 24 hours at 37° C., and then examined.

In tabulating the results the graphic method recommended by Morgenroth is employed. In the text-figures each square denotes one experimental animal; a black square indicates death from pneumococcal septicemia; a white square, survival; an oblique cross denotes that the animal died, but that the examination of the heart's blood was negative, in which instance death was apparently caused by the toxicity of the drug, the infecting pneumococci having been first killed off by its action;[1] and, finally, a square subdivided into

[1] Morgenroth pointed out that the toxic dose for mice is not far removed from the curative dose.

six smaller ones denotes that the animal was sick at the time of observation (Text-fig. 1).

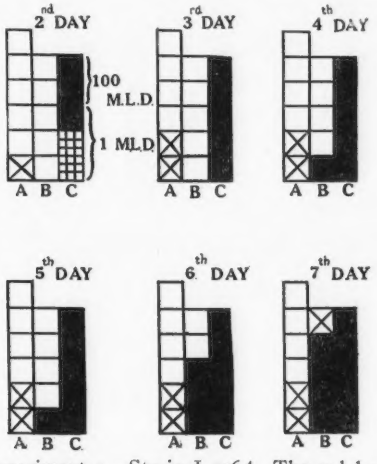


TEXT-FIG. 1. Explanation of the method used in the text-figures.

The condition of the animals is first given in the diagram on the second day of the experiment; that is, 24 hours after the infection and first treatment; consequently, the first diagram in each experiment is marked 2d day.

The minimum lethal dose of the infecting microorganism was in each case determined for forty-eight hours unless otherwise stated; in all cases that dose was taken as the m. l. d. which was with certainty fatal within the time limit stated for several mice. In case of doubt reestimations were made. With this statement it is unnecessary to give the estimations in detail.

Throughout the observations the mice were kept at about 75° F.[2]

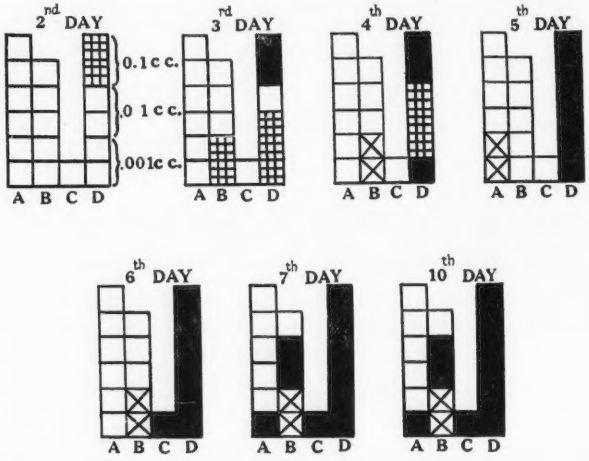


TEXT-FIG. 2. Experiment 1. Strain I 106.[4] The m.l.d. was 0.000,000,001 cc. (48 hrs.). The infecting doses used: 10 m.l.d. (Column A); 1,000 m.l.d. (Column B). Column C, controls (untreated).

[2] The toxicity of the drug is said to be greater when the animals are kept exposed to cold.

Comment on Experiment 1.—(Text-fig. 2.) Of the 6 treated animals infected with 10 times the m. l. d., 2 died of toxicity, the heart's blood at autopsy being sterile; while the remaining 4 animals remained permanently well; of those infected with 1,000 times the m. l. d. and treated, 1 died of toxicity (showing sterile heart's blood at autopsy), and the remaining 4 of pneumococcal septicemia; the last mentioned 4 animals, however, survived much longer than the untreated controls.

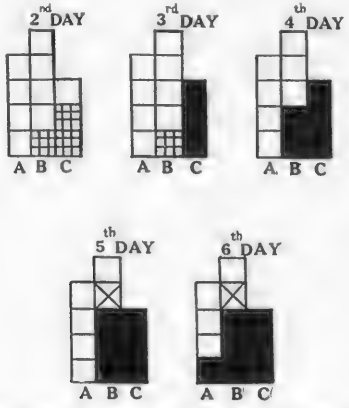
This microorganism was of extraordinarily high virulence for mice, it having had 106 animal passages. The m. l. d. above stated was regularly fatal and showed 6 to 14 colonies when plated.



TEXT-FIG. 3. Experiment 2. Strain I (B42)I[6]. The m.l.d. was 0.001 cc. The infecting doses used: 10 m.l.d. (Column A); 100 m.l.d. (Column B); 500 m.l.d. (Column C). Column D, controls (untreated).

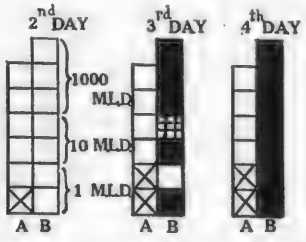
Comment on Experiment 2.—(Text-fig. 3.) This strain was relatively much less virulent than the previous one. A dose of 0.001 cc. or 0.01 cc. was always fatal within 4 to 5 days, but sometimes the animals infected with the larger of these doses survived those infected with the smaller by a day. In view of the protracted course of the infection in this case the time limit for the m. l. d. was judged as 5 days. Of those animals injected with 10 times the m. l. d. only 1 died of pneumococcal septicemia, all the others

surviving. Of those injected with 100 times the m. l. d. 1 survived; 2 died of toxicity; and 2 of pneumococcal septicemia, but later than the controls.



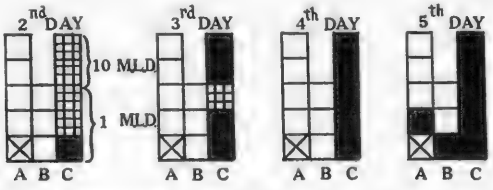
TEXT-FIG. 4. Experiment 3. Strain II 34^{19}. The m.l.d. was 0.000,000,001 cc. (48 hrs.). The infecting doses: 10 m.l.d. (Column A); 100 m.l.d. (Column B). Column C, controls (untreated).

Comment on Experiment 3.—(Text-fig. 4.) Of the 4 animals infected with 10 times the m. l. d. and treated, 1 died on the 6th day of pneumococcal septicemia and 3 survived. Of the 5 infected with 100 times the m. l. d. and treated, 3 died of pneumococcal septicemia and 1 survived; the remaining animal died of toxicity. On plating the m. l. d., 6 to 12 colonies resulted. This strain was one of very high virulence, notwithstanding the fact that it had undergone nineteen artificial cultivations since the last animal passage.



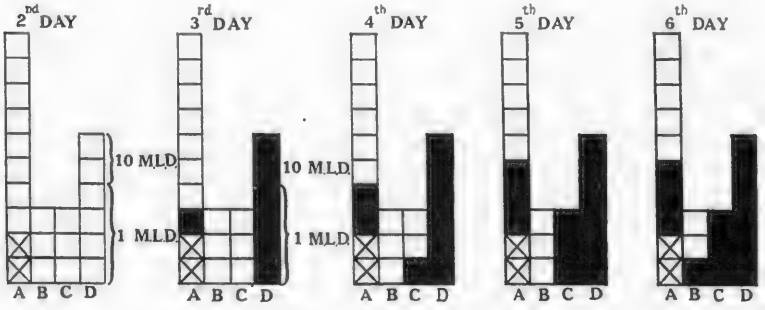
TEXT-FIG. 5. Experiment 4. Strain II (B21) 3^{2}. The m.l.d. was 0.0,000,001 cc. (fatal in 4 days). Dose used: 1,000 m.l.d. (Column A). Column B, controls (untreated).

Comment on Experiment 4.—(Text-fig. 5.) If the m. l. d. is judged to kill in 4 days, 4 mice out of 6 infected with 1,000 times the m. l. d. survived the controls permanently. Of the 2 given in Text-fig. 5 as dying of toxicity the heart's blood of 1 was sterile, and a Gram-negative bacillus was recovered from that of the other, but no pneumococci.



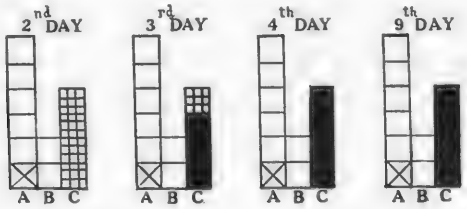
TEXT-FIG. 6. Experiment 4 a. Strain II (B21) 2^{14}. The m.l.d. was 0.01 cc. (48 hrs.). Doses used: 1 m.l.d. (Column A); 50 m.l.d. (Column B). Column C, controls (untreated).

Comment on Experiment 4 a.—(Text-fig. 6.) In this experiment 1 animal represented in Column A (1 m. l. d.) and 1 in Column B (50 times the m. l. d.) died on the 8th and 10th days, respectively, the heart's blood showing on examination a Gram-negative bacillus but no pneumococci. As we were troubled with mouse typhoid at this time, this was probably the cause of death. 5 animals survived, 2 died of pneumococcal septicemia, and 1 of toxicity.



TEXT-FIG. 7. Experiment 5. Strain III (A66) 12^{6}. The m.l.d. was 0.000,000,001 cc. (48 hrs.). Doses used: 10 m.l.d. (Column A); 100 m.l.d. (Column B); 1,000 m.l.d. (Column C). Column D, controls (untreated).

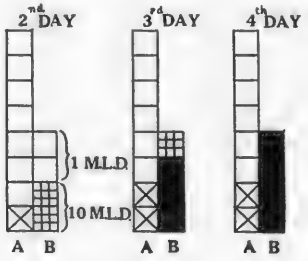
Comment on Experiment 5.—(Text-fig. 7.) The Strain III (A 66) 12^6 was a very virulent one. The m. l. d., 0.000,000,001 cc. of a 24 hour broth culture, when plated on blood agar, showed on incubation at least 15 typical colonies. The animal represented by the upper segment of Column C was found almost completely eaten by its fellows on the 6th day; no autopsy could be done, consequently the cause of death is somewhat doubtful. After the 6th day no further deaths occurred in this series. 5 out of 10 animals injected with 10 times the m. l. d. survived; 3 died of pneumococcal infection, and 2 of toxicity (giving a sterile heart's blood). Of 3 injected with 100 times the m. l. d. 2 survived, and the remaining animal succumbed to the infecting pneumococci. Of those injected with 1,000 times the m. l. d. none survived.



TEXT-FIG. 8. Experiment 6. Strain III (E22) 12^6. The m.l.d. (0.000,001 cc.) was fatal in 48 hrs. 0.00,000,001 cc. of a broth culture was observed to be regularly fatal within four days; the m.l.d. was not, however, at the time of this experiment determined for a longer period than 48 hrs.; consequently the dose of 0.000,001 cc. was probably considerably greater than the m.l.d. for a mouse. Column A, 1 m.l.d. (0.000,001 cc. (48 hrs.)). Column B, 10 m.l.d. (0.00,001 cc. (48 hrs.)). Column C, controls, 1 m.l.d. (untreated).

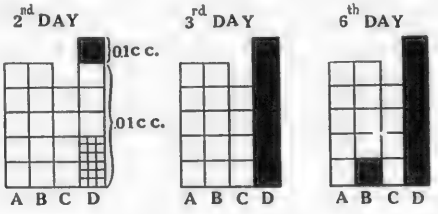
Comment on Experiment 6.—(Text-fig. 8.) Among the treated animals no death occurred up to the 10th day when observation was discontinued. 1 animal of the series died of toxicity, and none of pneumococcal septicemia.[3]

[3] Further experience with this strain has shown it to be more susceptible to the action of ethylhydrocuprein *in vivo* than any other strain of pneumococcus examined by us. We have been able with the drug to protect against 10,000 times the m.l.d.



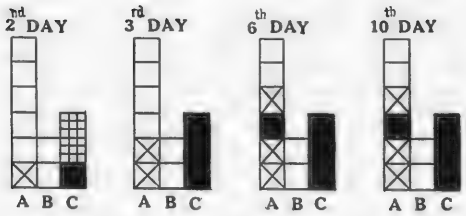
TEXT-FIG. 9. Experiment 7. Strain IV M.4[3]. The m.l.d. was 0.00,000,001 cc. of a 24 hr. broth culture. Dose used: 10 m.l.d. (Column A). Column B, controls (untreated).

Comment on Experiment 7.—(Text-fig. 9.) No further deaths occurred after the 4th day. Of 8 animals injected with 10 times the m. l. d. and treated, 2 died of toxicity and 6 survived. This was an unusually virulent strain of Group IV. The virulence, however, rapidly fell off on artificial cultivation.



TEXT-FIG. 10. Experiment 8. Strain South Africa (9).5[2]. The m.l.d. was 0.01 cc. Doses used: 10 m.l.d. (Column A); 20 m.l.d. (Column B); 50 m.l.d. (Column C). Column D, controls (untreated).

Comment on Experiment 8.—(Text-fig. 10.) Of the mice infected with 10 times the m. l. d. and treated all survived; of those infected with 20 times the m. l. d. and treated only 1 died of pneumococcal septicemia, all the others surviving; all those infected with 50 times the m. l. d. and treated survived. The controls infected with 1 m. l. d. all died within 48 hours.



TEXT-FIG. 11. Experiment 9. Strain IV (S 10)3^{10}. The m.l.d. was 0.01 cc. (48 hrs.). Dose used: 10 m.l.d. (Column A); 50 m.l.d. (Column B). Column C, controls, 1 m.l.d. (untreated).

Comment on Experiment 9.—(Text-fig. 11.) Of the 5 animals infected with 1 m. l. d. and treated 2 survived, and 3 died of toxicity. From the heart's blood of one of these latter a Gram-negative bacillus was recovered. One died of pneumococcal septicemia. Both the animals infected with 50 times the m. l. d. and treated recovered.

DISCUSSION.

The results of the present study agree, in the main, with those of the other workers mentioned. We have not had in our experiments such a large percentage of cures as is claimed by Morgenroth, namely 90 to 100 per cent; nor have we seen any definite protective action of optochin *in vivo* with an amount of infection greater than 1,000 times the m. l. d. of a highly virulent strain.[4] Moreover, the greater the virulence of the strain (by passage through mice) the greater was the difficulty in protecting against increasing multiples of the m. l. d. Constant results are difficult to obtain in the mouse, owing to the repeated injections apparently causing the animals a considerable degree of traumatism and consequently rendering them liable to intercurrent troubles. This was true of an epidemic of mouse typhoid which tended to obscure the results. The relative toxicity of the drug for mice is another factor which tends to render results not quite clear. Olive oil, the vehicle in which the drug is given, does not undergo absorption for a week or more, and the disturbance to the tissues caused by the repeated injections of this inert body seems to afford

[4] See page 281, footnote 3.

a suitable nidus for the growth of bacteria which may kill the animal before the observation is concluded. It seems likely that the detrimental factors, toxicity of the drug, the traumatism inflicted by the injections, and the effect of the infection itself, summate in their effects and thus render the probability of the animal surviving less likely, although its body may have been completely sterilized from pneumococcal infection. The effect of ethylhydrocuprein *in vitro* and *in vivo* on the pneumococcus is considerable and specific and is seen on type strains of all four groups of pneumococci.

From the text-figures it is seen that, of 85 mice infected with 100 times the m. l. d. or less and treated, 15 mice, or 17.6 per cent, died of pneumococcal septicemia; 13 mice, or 15.2 per cent, died of toxicity of the drug or some obscure cause, the heart's blood being sterile at autopsy (the corresponding controls invariably died of pneumococcal septicemia); 56 mice, or 66.8 per cent, survived. Of these 85 treated mice, 69, or 81 per cent, either recovered or died of causes other than pneumococcal septicemia; such, for example, as the toxicity of the drug.

CONCLUSIONS.

1. Ethylhydrocuprein hydrochloride in very high dilution inhibits the growth of, and in 18 hours kills, representatives of all four groups of pneumococci *in vitro*. The killing effect is generally seen in somewhat lower dilutions than the inhibiting effect. No constant or considerable difference is seen in these actions on representatives of the four groups of the pneumococci. The action of ethylhydrocuprein hydrochloride on the pneumococci *in vitro* is so strongly specific that it may possibly be used as a test for a true pneumococcus.

2. The inhibitory or killing effects of ethylhydrocuprein hydrochloride *in vitro* on bacteria other than pneumococci are slight or absent. The effects are greater on streptococci than on any other organisms examined, but are still much less than on the pneumococci. This action distinguishes between the streptococcus group, including *Streptococcus mucosus* sometimes found in normal

mouths, on the one hand, and the true pneumococcus (including *Pneumococcus mucosus*), on the other.

3. Quinine hydrochloride inhibits the growth of, and kills the pneumococcus *in vitro;* much stronger concentrations, however, are necessary than in the case of ethylhydrocuprein. This effect of quinine hydrochloride is also seen on other organisms, but in a less degree.

4. Ethylhydrocuprein (optochin base) has a well marked protective action against experimental pneumococcal infection in mice in the case of type strains of all four groups of pneumococci; this protective action may be efficient against many multiples of the minimum lethal dose.

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THE EFFECT OF IRRITATION ON THE PERMEABILITY OF THE MENINGES FOR SALVARSAN.

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(Received for publication, June 9, 1915.)

One of the explanations offered for the beneficial effects of intraspinal injections in the treatment of syphilis of the central nervous system is that there may thus be induced an increased permeability of the meninges following their irritation by the injected substance. The purpose of this investigation was to determine whether the intraspinal, or rather subdural, injection of various substances used in intraspinal treatment would increase the amount of arsenic in the cord and brain of animals which received intravenous injections of salvarsan at the same time.

Sicard and Reilly (1) described a method for increasing the permeability of the brain substance for drugs by trephining the skull and injecting 5 cc. of a 0.5 per cent solution of sodium chloride under the dura with a fine hypodermic needle. They showed that in the cadaver a similar amount of India ink injected subdurally was distributed over an area 8 or 10 cm. in diameter, and suggested from this experimental evidence that two subdural injections of saline, *i. e.*, one in each temporofrontal region, would be sufficient to increase the permeability of a large part of the cerebrum for salvarsan introduced intravenously. They also state that they have injected 0.1 mg. of cyanide of mercury subdurally in the frontal region without harmful effect, and on the following day given salvarsan or neosalvarsan intravenously to the same patient. The cyanide of mercury was used simply to increase the permeability of the tissues. Viton (2) states that after a slight chemical irritation the meninges are rendered more permeable, and he used for this purpose a preparation suggested by Sicard (3) which consists of cyanide of mercury 0.1 mg., and novocain 0.015 gm. in 2 cc. of a 0.5 per cent saline solution. The intraspinal injections were made at intervals of 1 month, mercury or salvarsan being given in the usual way. He states that with this method of treatment the irritative symptoms of tabes are much relieved. Tinel and Leroide (4) injected several drops of a 1 per cent solution of sodium nucleinate into the fourth ventricles of rabbits and immediately afterwards gave the animals 10 cg. of neosalvarsan intravenously. One hour later they again punctured the fourth ventricles and obtained 4 cc. of fibrinous fluid,

which contained 0.008 mg. of arsenic. Fluid from the fourth ventricles of control animals contained no arsenic. The fibrinous fluid was evidence of a much more intense irritation than is usually produced by subdural injections in patients, so the increase in permeability which these authors demonstrated cannot be applied to bedside treatment.

The intraspinal injection of normal salt solution, or serum, does produce, however, a temporary irritation of the meninges, as is shown both clinically by pains, and by examination of the cerebrospinal fluid a few hours after such an injection, when several hundred cells per cmm. may be found. It is reasonable to suppose that this irritation might increase the permeability of the tissues, and that salvarsan circulating in the blood would be deposited in larger amounts in the tissues contiguous to the irritated meninges, than when the meninges are in their normal impermeable condition.

Methods.

To determine whether a demonstrable increased deposition of arsenic actually does occur, the following experiments were performed. Cats were selected as the experimental animals, for the following reasons: Intravenous injections can be easily given into the marginal vein of the ear, and there is a fairly large subdural cistern surrounding the cauda equina, so that subdural injections in the cat are similar in nature to those in man in that the injecting needle is not brought into direct contact with the cord. In rabbits the cord extends so low that it is impossible to introduce a needle under the dura and inject a solution without injuring the cord. In the experiments under discussion it was necessary that the nervous tissue be injured in no other way than that possibly resulting from the presence of the substance injected. The intraspinal injections in the cats were made in the following manner: The animals were etherized, and the dura was exposed by laminectomy of the two lower lumbar vertebræ. A fine curved hollow needle attached to a syringe was introduced through the dura, and cerebrospinal fluid was aspirated into the syringe, which was then detached, and another syringe containing the solution to be injected was attached to the needle, and the solution slowly injected. The injections were always preceded by aspiration of cerebrospinal fluid, to be sure that the needle was properly placed. All the animals were

injected intravenously with salvarsan in alkaline solution in the proportion of 0.05 of a gram per kilo of body weight. Some of the animals received the intravenous injections one hour before the intraspinal treatment, and in others the order was reversed, so that at the time of intravenous injection the meninges would be already in a state of irritation. On the following day the animals were exsanguinated by opening the jugular veins and carotid arteries, in order to remove as much arsenic-containing blood from the tissues as possible. The cord and brain were immediately removed from the body and the brain was divided into three portions: cerebrum, cerebellum, and midbrain, pons, and medulla; the cord was separated from the medulla, and all the specimens were dried separately. The dried tissue was powdered, thoroughly mixed, and weighed, and duplicates of each specimen were analyzed quantitatively for arsenic.

The analyses were made by a special method devised for the purpose by Vinograd (5). This method depends for its accuracy upon the principle of oxidizing the tissues with small amounts of arsenic-free nitric acid, at 260° C. in a sealed glass bomb. The bomb is then opened, sulphuric acid added, the nitric acid driven off by heating, and the sulphuric acid-arsenic mixture quantitated for arsenic by Sanger and Black's modification of Gutzeit's method. All reagents and utensils were carefully tested for arsenic before using. The standard scales for comparison were made with both arsenious acid and salvarsan. In the results here presented the figures are given in fractions of mg. of salvarsan per gram of dried tissue.

RESULTS.

The operative procedures, substances injected, time relations, and results of the analyses are given in Table I. Four control animals were first treated. Two of them had only intravenous injections of salvarsan. One of these killed one and one half hours later showed no more arsenic in its cerebrospinal axis than one killed after eighteen hours. The comparatively slight neurotropic action of salvarsan demonstrated by Ullmann (6) and by Stühmer (7) probably explains this similarity in arsenic content at such different intervals after treatment. In the other two controls the operative procedure, laminectomy and withdrawal of cerebrospinal fluid, was shown to

have no effect. The effect of irritation with the following substances was studied: isotonic salt solution; 50 per cent cat serum diluted with isotonic salt solution; pure cat serum; salvarsanized cat serum obtained by treating cats with 0.05 of a gram of salvarsan per kilo of body weight, bleeding one hour later, separating the serum, and heating to 56° C. This serum contained between 0.015 and 0.025 mg. of salvarsan per cc. of serum. It was injected in 50 per cent dilution and also undiluted. A mixture of cyanide of mercury, novocain, and 0.5 per cent saline, similar to that used by Sicard and by Viton, was also injected.

As a rule, the cords of the ten animals which received intraspinal injections contained no more arsenic than the four controls. The two exceptions are Animals H 104 and H 106, which received intraspinal injections of normal cat serum in a 50 per cent dilution. There are two possible explanations for this variation. First, the 50 per cent serum may have been more irritating than the other substances injected; or, second, the animals were not so completely exsanguinated. We are inclined to attribute the increased amount of arsenic in the cords of these animals to incomplete exsanguination, for in both animals the cerebrum and cerebellum contained considerably more arsenic than the average. Furthermore, if the 50 per cent serum were the important factor, one would expect that the cords of Cats H 110 and H 112, which received intraspinal injections of 50 per cent dilution of salvarsanized serum, would show a similar increase in arsenic, but in these animals both the cords and brains showed the average arsenic content. Upon first thought, one would expect that the cerebrospinal axis of the animals that received subdural injections of salvarsanized serum would contain more arsenic than the animals injected with normal serum or mercury. The rapid diffusion of the small amount of arsenic in the serum through the cerebrospinal fluid, and the rapid excretion of substances from the cerebrospinal fluid into the blood stream, probably explain the fact that the cords and brains of animals which received salvarsanized serum intraspinally contained no more arsenic than the average. Both of these factors have been conclusively demonstrated by Dandy and Blackfan (8). Hall (9) has also shown that the cerebrospinal fluid of patients who received

TABLE I.

Animal No.	Weight.	Operative procedure.	Substance injected intraspinally.	Time relation of intraspinal injection (I.S.) to intravenous injection (I.V.).	Killed after intravenous injection.	Salvarsan per gm. of dried tissue.				Remarks.
						Cerebrum.	Cerebellum.	Midbrain, pons, medulla.	Spinal cord.	
	gm.				*hrs.*	*mg.*	*mg.*	*mg.*	*mg.*	
H 115	3,000	None	None		1½	0.025	0.025	0.025	0.017	
H 113	2,560	"	"		18	0.017 (N)*	Traces	0.025	0.025 (N)*	
H 105	3,000	Laminectomy	"		19	0.037	0.037	0.025	0.025	
H 107	1,080	Laminectomy and 1 cc. cerebrospinal fluid withdrawn	"	1 hr. before I.V.	18	0.025	0.025	0.017	0.017	
H 116	3,100	Laminectomy and subdural injection	1 cc. 0.9% sodium chloride solution	I.S. 1 hr. after I.V.	17	0.017	Traces	0.025	Traces	
H 104	2,800	" "	1 cc. 50% normal cat serum	" " before "	13	0.037	0.037	0.025	0.075	
H 106	1,900	" "	" "	" " after "	17	0.075	0.075	0.025	0.037 (N)*	
H 109	1,550	" "	1 cc. 100 % normal cat serum	" " before "	16	0.025	0.017	0.025	0.025	
H 108	2,050	" "	" "	" " after "	16	0.025 0.025	Traces	0.025 0.017	0.025	Weakness in both hind legs day after operation.
H 112	2,650	" "	1 cc. 50% salvarsanized cat serum	" " before "	15	0.017	0.025	0.017	0.025	
H 110	2,250	" "	" "	" " after "	19	0.017	0.017	Traces	0.025	Weakness in right hind leg day after operation.
H 111	1,670	" "	1 cc. 100% salvarsanized cat serum	" " " "	19	0.025	Traces	0.017	0.025	
H 117	2,500	" "	Mercury cyanide solution	" " before "	19	0.025	0.017 (N)*	0.025	0.025	Weakness in both hind legs day after operation.
H 118	2,850	" "	" "	" " after "	16	0.017	Traces	Traces	0.025	

* (N), duplicate lost. All results are in duplicate unless otherwise noted.

intraspinal injections of neosalvarsan often contains no arsenic twenty-four hours after the injection.

If irritation alone were the important factor in determining the deposition of arsenic in the nervous tissue, the cords, which were more exposed to the irritating effect of the substances injected than the brains, should have shown a higher arsenic concentration. The average arsenic content of the cord and of the cerebrum was practically the same; *i. e.*, 0.029 mg. for the former, and 0.028 mg. for the latter. The cords of the three animals, H 108, H 112, and H 113, which showed clinical evidences of cord or cauda equina injury did not contain more arsenic than the cords of animals which showed no evidence of local lesions. Nor did the more irritating mercury solutions increase the arsenic in the cords or brains of the animals which received it. There was no difference between the animals that received intraspinal injections an hour before the intravenous injections of salvarsan, and those that first received the salvarsan intravenously.

CONCLUSIONS.

The subdural injection of normal salt solution, normal serum, serum salvarsanized *in vivo* or weak solutions of cyanide of mercury does not demonstrably increase the permeability of the spinal cord or brain for salvarsan which is circulating in the blood at the time of the subdural injection.

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CHANGES IN THE ELECTROCARDIOGRAMS ACCOMPANYING EXPERIMENTAL CHANGES IN RABBITS' HEARTS.

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Plates 37 to 40.

(Received for publication, June 14, 1915.)

Differences in electrical potential are developed in the structures of the heart during the processes of its action. At any instant of time the waves of the electrocardiogram are the resultant of a number of potential differences developed by the synchronous activities of the cardiac structures. It has been sufficiently demonstrated that the P wave is associated with activity of the auricles, and the Q, R, S, T, and U waves with activity of the ventricles. Clement (1), Erfmann (2), and Lewis (3) have recently shown that the Q, R, and S waves are associated with the spread of the excitation wave from the auriculoventricular junction to the ventricles and throughout the ventricular muscle mass. It is unknown what ventricular structures are associated with the individual waves. It is also unknown whether the direction of the potential difference responsible for a given wave corresponds to any anatomical structure. On theoretical grounds Einthoven (4) and Waller (5) have shown that from the relative sizes of a given wave in the three leads of the human electrocardiogram it is possible to calculate the angle which the direction of potential difference responsible for the wave makes with the anatomical axis of the body. Theoretically then, alterations in the position of the heart in the body that change the direction of the potential difference relatively to the axis of the body will change the size and direction of the waves. Again, if without any change in the position of the heart, alterations occur within the heart so that the distribution of the muscle mass is changed, as for

instance by a change in the thickness of the wall of one ventricle relatively to the wall of the other, or by a deviation of the septum to one side or the other, changes in the resultant potential difference would result with corresponding changes in the size and direction of the waves. Other factors are involved in determining the potential differences and character of the waves. These include the direction and rate of travel of the wave of excitation within the conducting structures and the muscle mass.

On account of these considerations it is generally recognized that when changes in the electrocardiogram occur they do so only as the result of definite causes. Instances of such changes are observed in hypertrophy of the human heart.

In patients with hypertrophy of the left ventricle, the R wave is large in Lead I and small or absent in Lead III, while the S wave is large in Lead III and small or absent in Lead I; and in patients with hypertrophy of the right ventricle, the R wave is small or absent in Lead I and large in Lead III, while the S wave is large in Lead I and small or absent in Lead III. These changes correspond to the changes expected theoretically, if in hypertrophy the direction of potential difference responsible for the Q, R, S group is turned to the left or to the right respectively. Other changes have been observed in the course of acute and chronic illnesses; as, for instance, alterations in the size of all or of some of the waves, splitting of waves, and lengthening of the time occupied by a wave or group of waves. These changes are often transitory and so must depend on causes other than increased muscle mass.

The work reported here was undertaken originally in the hope of correlating certain changes in the electrocardiogram with definite changes in the heart.

Method and Material.

For this purpose it was necessary to record changes in the heart during life. For observations on the position, size, and shape of the heart, x-ray plates were made. It was impossible to determine changes in the distribution of the muscle during life. For this and for the condition of the cardiac structures it was necessary to depend upon the examinations made after death. The postmortem examinations were satisfactory for these purposes only in the animals that

died or were killed at the time when the electrocardiographic changes were observed. After death the hearts were divided as recommended by Müller (6), and the chambers weighed separately. It was not found advisable to separate the two auricles and auricular septum from each other. Histological examinations were then made.

Rabbits were used in all the experiments. Full growth rabbits were chosen so that changes consequent on growth could be excluded, as it was found necessary to continue the observations in some cases for a period of six months or more.

In all, 33 rabbits were used, of which 4 were kept as controls. The changes were produced by repeated intravenous injection of: (a) spartein sulphate and adrenalin hydrochloride in 7 animals; (b) adrenalin hydrochloride alone in 4; (c) diphtheria toxin in 4; (d) suspensions of living streptococci in 14 animals.[1]

Spartein sulphate and adrenalin hydrochloride were used in the doses recommended by Fleisher and Loeb (7), and the injections were repeated at intervals of three or more weeks. Adrenalin alone was given in increasing doses every two days commencing with 0.1 or 0.2 cc. of 1:1,000 solution for twenty or more doses. Diphtheria toxin was given in varying doses and these were repeated if no definite effect was seen. The streptococci which were injected were isolated from the joints and blood of cases of acute rheumatism, from a tonsillar abscess, a liver abscess, and an abscess about the root of a tooth. Some were hemolytic, others of the *Streptococcus viridans* type. The injections were repeated, as a rule, in increasing doses until effects on the animal or electrocardiographic changes were seen.

The roentgenograms were made with the anticathode of the tube at a distance of 30 inches from the lower end of the sternum. The rabbit was stretched out on its back on a board, in which a gap had been cut so that the plate was in contact with the back of the animal.

Before injection, control electrocardiograms were made with the animal on the right side and on the left side, as well as on its back, so that possible alterations due to changes in the position of the heart

[1] The animals injected with streptococci were from a series of experiments undertaken with Dr. Homer F. Swift. The organisms were isolated and the injections made by him.

in the body could be estimated. In only four instances were changes in the size of the waves of more than 1 mm. noted with change of position.

The large Edelmann model of the string galvanometer was employed. On all occasions three leads were recorded. In most cases Lead I (right foreleg and left foreleg) was unsatisfactory, as the waves were very small; and Lead III (left foreleg and left hindleg) was, as a rule, so nearly identical with Lead II (right foreleg and left hindleg) that it has been found sufficient to consider Lead II only.

The resistance of the animal was estimated by the Wheatstone bridge and telephone method, and the string standardized with a constant total resistance in the circuit. The electrocardiograms were made with a resistance added to bring the total resistance in the circuit to a constant value. The deflection times of the different strings employed varied and the times occupied by some of the waves of the rabbits' electrocardiograms are so short that the size of the wave was affected by the variations in deflection time. It has been necessary, therefore, to discard all records in which the deflection time of the string varied from that of the control curves. Records in which the standardization of the string was not satisfactory have also been discarded. More rapid strings than those available might have shown greater changes than have been observed by the methods employed, but such changes as have been recorded would have been shown and shown better by an improvement in the methods.

RESULTS.

The changes in the electrocardiograms to be described are those involving the Q, R, S group only. Changes in the P and T waves also occurred but were so small, except in a very few instances, that accurate comparisons were impossible.

In the course of the observations on the 7 animals injected with spartein and adrenalin the roentgenograms in three animals showed that extensive dilatations to the right or to both sides developed in the course of a few days. Dilatations to the left were accompanied by decrease in the size of the R waves and increase in the size of the S waves; the one instance of marked dilatation to the right by

increased R wave and diminished S wave; while dilatation to both sides caused little or no change. These acute changes disappeared as quickly as they developed. Of the other 4 animals, 2 died after the first injection, and 2 after the second, and before significant changes developed. After repeated injections and the resultant acute changes, these 3 animals showed a moderate gradual enlargement to the left associated with decrease in the size of the R waves and increase in the S waves.

Figs. 1 and 3 represent the changes that occurred in Rabbit 218 E. Fig. 1 shows the size of the heart at the beginning, and Fig. 3 at the conclusion of the experiments, and they demonstrated an enlargement to the left side. Figs. 2 and 4 show the corresponding electrocardiographic changes, and Fig. 5 shows the extreme but transitory electrocardiographic changes obtained in this animal. Unfortunately no roentgenogram was obtained on the same day as this curve.

Figs. 6 and 8 represent the right-sided enlargement that occurred in Rabbit 211 E, and Figs. 7 and 9 represent the change in the electrocardiograms. Figs. 10 and 12 represent the extreme change obtained in Rabbit 278 E and Figs. 11 and 13 give the corresponding electrocardiograms. The enlargement in this case was to both sides while the electrocardiogram was altered but little. In Table I the changes in the waves and in the size of the heart throughout the observations in Rabbit 278 E are tabulated. It will be seen that following the third injection the electrocardiographic changes indicated an enlargement to the left side, but no roentgenogram was obtained on that date to correspond to this. Similar changes occurred after the last injection. Daily x-ray observations at this time showed an enlargement to the left.

Of the 4 animals treated by repeated injection of adrenalin alone, one, 106 B, showed an acute enlargement to the left accompanied by increased R wave and increased S wave, but died after the 4th injection, while the others showed slight gradual increase to the left with similar electrocardiographic changes.

Of the animals injected with adrenalin, or spartein and adrenalin, those that showed marked distress immediately after injection developed acute changes, while in those that showed little immediate

TABLE I.*

Rabbit 278 E.

Received 6 Injections of Spartein Sulphate 0.012 Gm. per Kilo, and Adrenalin Hydrochloride 0.2 Cc. 1: 1,000 (Parke, Davis and Co.). Total Resistance in Circuit 13,500 Ohms throughout Observations. String Standardized with Resistance in Circuit of 8,700 Ohms.

Date.	Injection.	Rate.	Deflection time.	Size of waves. Q	R	S	Size of heart.	Remarks.
			sec.	*mm.*	*mm.*	*mm.*	*mm.*	
Dec. 2, 1913		267.3			+3.5	−1.0		On back.
" " "		266.4	0.020		+3.0	−1.0		On right side.
" " "		272.7			+2.5	−1.0		On left side.
" 4, "							28 x 23	
" 8, "	1							
" 12, "		246.7	"		+2.5	−1.0		
" 19, "		281.8	"		+2.0	−1.0		
" 30, "		284.3	0.025		+2.5	−3.0		
Jan. 7, 1914		267.5	"		+1.5	−1.5		
" " "	2							
" 12, "		293.7	"		+2.5	−2.0		
" 20, "		216.9	0.015		+2.0	−1.0		
" 22, "							32 x 28	
" 26, "		235.2	0.018		+2.5	−2.0		
" " "	3							
" 29, "		259.2	0.025	− ?	+ ?	−1.5		
Feb. 3, "		261.8	"			−3.5		
" 10, "		274.5	"		+0.5	−2.0		
" 26, "		267.1	0.020		+1.5	−2.0		
Mar. 5, "							38 x 32	
" 17, "		235.4	"		+2.0	−2.0		
" " "	4							
" 23, "		289.1	"		+3.0	−1.0		
" 25, "		273.1	"		+2.0	−0.5		
" 30, "		266.9	"		+2.0	−1.0		
Apr. 6, "							53 x 44	Enlarged to both sides.
" 7, "		261.7	"		+2.0	−1.0		
" " "	5							
" 10, "		255.6	"		+2.0	−1.0		
" 15, "							39 x 33	
" 16, "		230.8	"		+2.0	−2.0		
" 27, "							39 x 31	
" 30, "		233.8	"		+2.5	−2.0		
" " "	6							
May 1, "		254.2	"		+2.0	−1.5	38 x 32	
" 4, "		253.9	"		+1.5	−2.5	37 x 32	
" 5, "		240.0	"		+1.0	−2.0	36 x 33	
" 6, "							35 x 31	
" 7, "		238.4	"		+1.0	−3.0	35 x 30	
" 8, "		244.8	"		+1.0	−3.0	42 x 38	Enlarged to left mainly.
" 11, "		258.2	"		+1.0	−3.5	36 x 29	
" 13, "		268.2	"		+1.0	−2.5	34 x 26	
" 24, "		267.0	"		+1.0	−3.0		
" 25, "							34 x 25	
June 4, "		201.1	"		+2.0	−1.5		
" 5, "		234.8	"		+2.0	−1.5	33 x 25	Enlarged to left mainly.

* The figures in this table and in Table II denoting the size of the heart were obtained by measuring and expressing in mm. the long axis of the heart shadow and the greatest width at right angles to this.

reaction to the injections only slight changes developed in the size of the heart or in the electrocardiograms. In all cases the heart sounds became accentuated and reduplicated and in four cases systolic murmurs developed, but except for restlessness and increased excitability, the animals did not appear to be distressed after recovering from the dyspnea consequent on the injections. This dyspnea never continued for more than a few minutes.

The three animals injected with fatal doses of diphtheria toxin showed slowing of the heart rate before death. Not only did the auricular rate decrease but the auriculoventricular conduction time lengthened, and the time occupied by each phase of the cycle increased. For example, in Rabbit 103 B the heart rate changed from 261.7 to 177.6, the P–R time from 0.07 of a second to 0.10 of a second, the time occupied by the Q, R, S waves from 0.02 to 0.07 of a second, and that occupied by the T wave from 0.12 to 0.16 of a second. Rabbit 105 B, which received three non-fatal doses, showed by x-ray slight enlargement of the heart downwards and to the left with increase in the S wave and decrease in the R wave of the electrocardiogram. Histologically the cases that received fatal doses showed marked degeneration and fatty changes throughout the heart muscle and similar changes in the auriculoventricular bundle. The electocardiographic changes are illustrated in Figs. 14 and 15 from Rabbit 102 B.

No constant changes were seen in the fourteen rabbits that received intravenous injection of streptococci. Lesions resulting from these injections in structures other than the heart occurred but will not be discussed here. Rabbit 115 B was injected with a streptococcus obtained from the blood of a case of acute articular rheumatism. Slowing of the auricular rate occurred and slight lengthening of the auriculoventricular interval and of the Q, R, S time. The changes were similar to those seen in the cases injected with diphtheria toxin. Premature ectopic ventricular contractions also occurred before death. Histologically the heart muscle showed diffuse degenerative changes and numerous necrotic foci. Rabbit 118 B injected with a streptococcus isolated from a tonsillar abscess also developed premature ectopic ventricular contractions, but no focal lesions were found and the muscle showed but slight degenera-

tive changes. Figs. 16 and 17 illustrate the electrocardiographic changes in this animal. Rabbit 119 B injected with the same organism showed lengthening of the Q, R, S period. In the heart there were numerous areas of focal necrosis and degeneration of the muscle. Rabbit 108 B, injected with a *Streptococcus viridans* obtained at death from the heart's blood of a case of acute rheumatic carditis, showed on an x-ray plate an acute enlargement of the heart to the left; the accompanying electrocardiogram showed a decrease in size of all the waves, but especially of the R wave.

In 9 of the 14 rabbits treated with suspensions of streptococci and in all 4 of those receiving diphtheria toxin, diminution in the size of all the waves was seen. This was never found in the controls and only to a very slight extent in the animals treated with adrenalin or

TABLE II.

Rabbit 109 B.

Received 4 Injections of a Suspension of Streptococcus Isolated after Death from the Heart's Blood of a Patient with Acute Rheumatism. Total Resistance in Circuit for Standardization and for Records, 8,700 Ohms. Deflection Time, 0.02 Seconds throughout Observations.

Date.	Injection.	Rate.	P-R time.	Size of waves.				Q-R-S time.
				P	R	S	T	
			sec.	*mm.*	*mm.*	*mm.*	*mm.*	*sec.*
Mar. 20, 1914		170.8	0.07	+1.0	+4.0	−2.5	+3.0	0.025
" " "	1							
" 23 "		231.6	"	+1.5	+2.5	−3.5	+2.0	0.025
" 24 "		211.4	"	+1.0	+2.0	−2.5	+1.5	0.025
" 25 "		244.8	"	+1.0	+1.0	−2.5	+1.0	0.02
" 28 "		244.5	"	+0.5	+1.0	−1.0	+0.5	0.025
" 30 "		264.5	"	+1.0	+1.5	−2.0	+1.5	0.025
Apr. 2 "		230.7	"	+ ?	+1.0	−1.0	+0.5	0.02
" 6 "		253.3	"	+1.0	+3.5	−2.0	+2.5	0.025
" 8 "		228.8	0.06	+1.0	+3.5	−1.5	+1.5	0.02
" 14 "		256.8	"	+1.5	+3.0	−2.5	+2.0	0.025
" 24 "		216.3	0.07	+1.5	+5.0	−2.0	+2.5	0.025
" " "	2							
May 3 "		293.0	"	+1.0	+2.0	−3.0	+1.5	0.03
" 4 "		284.8	"	+1.0	+2.0	−1.0	+1.5	0.025
" 7 "		269.1	0.06	+1.0	+3.0	− ?	+2.0	0.025
" 11 "		238.4	0.07	+1.0	+3.0	−1.0	+2.0	0.025
" 18 "	3							
" 22 "		278.2	0.06	+1.0	+2.5	−1.0	+1.5	0.025
" 25 "		241.7	0.07	+0.5	+1.0		+1.0	?
" 27 "		212.3	"	+0.5	+2.0	−1.0	+1.5	0.025
" 28 "	4							
June 8 "		218.6	"	+1.0	+3.0	−1.5	+1.0	0.025

spartein and adrenalin, and in them it was a transient effect. Table II, giving the observations on 109 B, illustrates this.

DISCUSSION.

Changes in the size and direction of the waves of the electrocardiogram such as those described may depend, as has been pointed out, on changes in the position of the heart in the body, changes in the relative distribution of the muscle mass, and changes in the condition of the muscle and conducting structures. It is possible that conditions of the nervous system may exert an influence from outside the heart. But the influence of the nervous system on the mechanism of the heart beat has been the subject of numerous investigations and changes of this nature have never been demonstrated. The influence of the position of the heart in the body is definitely excluded, as control curves, taken with the animals in different positions before the injections were commenced, showed that in no instance could the changes that resulted be accounted for in this way. In some instances also it was ascertained that alterations in the position of the rabbit could not restore the electrocardiogram to the initial form. The changes in the size and shape of the heart demonstrated by the x-ray examination were always accompanied by changes in the electrocardiogram, and changes in one direction were always accompanied by electrocardiographic changes in a definite direction. It is probable, therefore, that the alterations seen in the roentgenograms were the causes of the electrocardiographic changes described.

The observations extended over seven months in some instances. The records were obtained at all stages between the initial normal forms of the electrocardiograms and the extreme qualitative changes usually associated with right- or left-sided enlargement. These records show that not only the extreme qualitative changes, but also the gradual quantitative decreases and increases in size of the waves of the Q, R, S group seen in the intermediate records are significant of changes in the size and shape of the heart.

Diminution in the size of all the waves was seen in the case of the animals treated with diphtheria toxin. The hearts of these animals showed extreme degenerative changes after death. Acute

degenerative changes have been described by numerous observers after single injections of adrenalin and of spartein and adrenalin. Following the injections, the electrocardiographic records sometimes showed decrease in size of all the waves before the changes associated with the enlargements of the heart manifested themselves and obscured these more general changes. A toxic condition of the muscle and other structures of the heart and degenerative changes in them must alter their functions, and it is not improbable that their electrical activities may be lowered. All the animals injected with streptococci showed joint lesions or altered heart sounds during life, or degenerative changes in the heart muscles after death, so that a similar reason may be present in the nine animals so treated that showed the decrease in the size of all the waves.

The influence of the nervous system and of pathological lesions of the structures themselves on the function of the pace-maker and the auriculoventricular conducting structures has been repeatedly demonstrated. The slowing of the pace-maker and the lengthening of auriculoventricular conduction time and of the duration of the individual waves, seen in the three rabbits treated with fatal doses of diphtheria toxin and in Rabbits 115 B and 119 B treated with streptococci, may have been caused by changes in the nervous mechanism outside the heart. But the intense degenerative changes seen throughout the conducting structures and the heart muscle afford a more probable explanation. Ectopic ventricular contractions can be caused by mechanical or electrical irritation of the heart muscle. The ectopic contractions seen in Rabbit 115 B may well have been due to the foci of degeneration and necrosis seen. Ectopic ventricular contractions were also obtained in Rabbit 118 B although no such foci were found in the sections examined.

A study of the relative weights of the ventricles of the hearts after death showed no changes sufficiently constant to permit of correlation with the electrocardiographic changes.

CONCLUSIONS.

1. In rabbits transient and also permanent enlargements of the heart occur as the result of the intravenous injection of adrenalin, spartein and adrenalin, diphtheria toxin, and streptococci.

2. Transient and permanent enlargements of the heart to the left are associated with decrease in the size of the upwardly directed waves of the Q, R, S group in Lead II of the electrocardiograms and increase in the downwardly directed. Transient enlargement to the right is associated with changes in the opposite direction.

3. The stages in the process of enlargement have been observed and are associated with gradual changes in the size of these waves.

4. A diminution in the size of all the waves of the Q, R, S group was observed in degenerative conditions of the heart muscle.

5. Extreme degeneration of the structures of the heart was associated with slowing of the heart's action, lengthening of conduction time (P–Q), and lengthening of the time occupied by the individual waves of the electrocardiogram.

6. Ectopic ventricular contractions were seen in rabbits injected intravenously with suspensions of living streptococci.

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EXPLANATION OF PLATES.

PLATE 37.

FIGS. 1 to 5. Rabbit 218 E. . Received injections of 0.012 gm. spartein sulphate per kilo and 0.2 cc. of adrenalin hydrochloride 1 : 1,000 (Parke, Davis and Co.) on Dec. 8, 1913, Jan. 2, Jan. 26, Mar. 17, Apr. 6, and Apr. 30, 1914.

FIG. 1. Roentgenogram of heart, Nov. 28, 1913.

FIG. 2. Electrocardiogram, Lead II, Dec. 4, 1913.

FIG. 3. Roentgenogram of heart, May 5, 1914.

FIG. 4. Electrocardiogram, Lead II, May 5, 1914.

FIG. 5. Electrocardiogram, Lead II, Feb. 3, 1914.

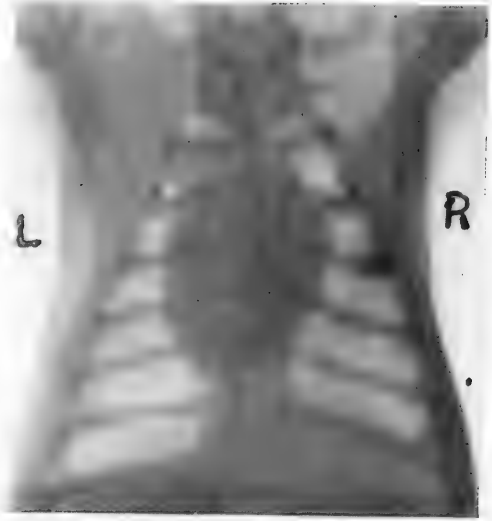

FIG. 1.

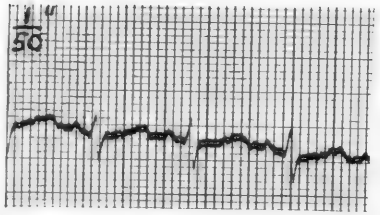

FIG. 2.

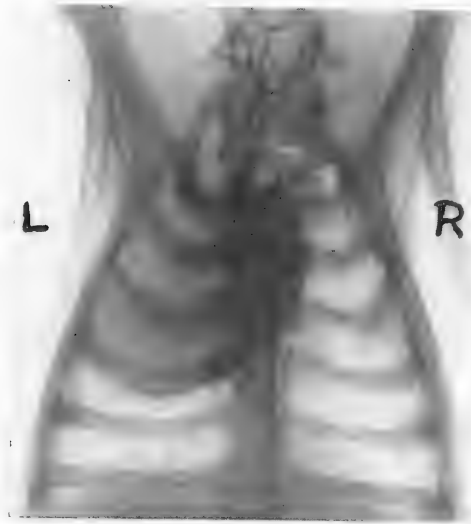

FIG. 3.

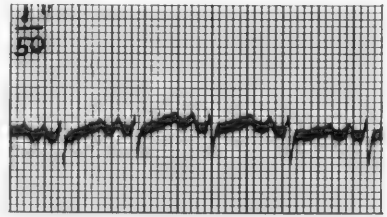

FIG. 4.

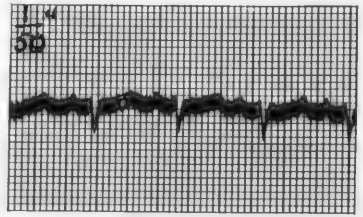

FIG. 5.
(Fraser: Changes in Electrocardiograms.)

FIG. 6.

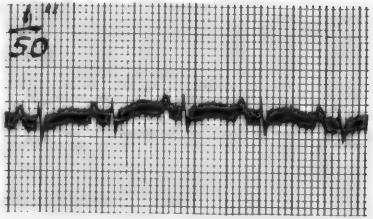

FIG. 7.

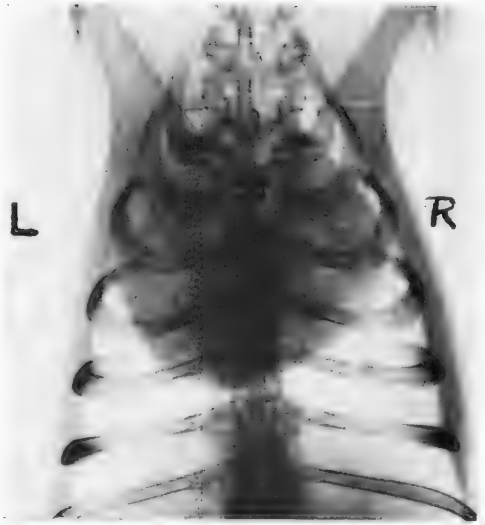

FIG. 8.

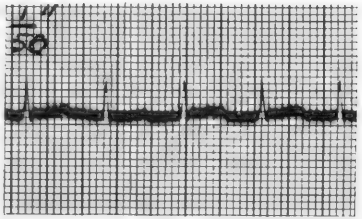

FIG. 9.

(Fraser: Changes in Electrocardiograms.)

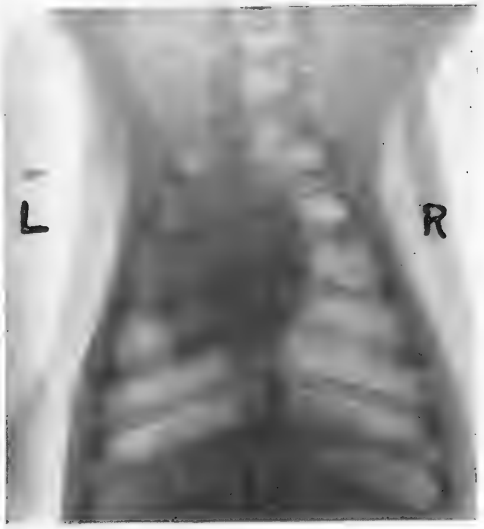

FIG. 10.

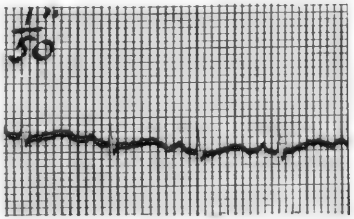

FIG. 11.

FIG. 12.

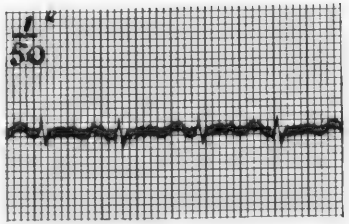

FIG. 13.

(Fraser: Changes in Electrocardiograms.)

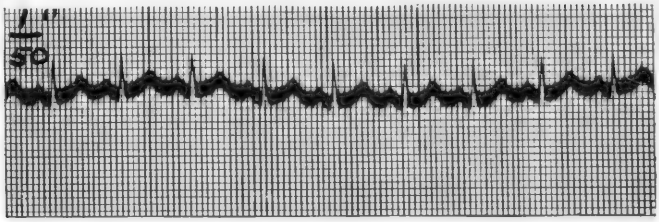

FIG. 14.

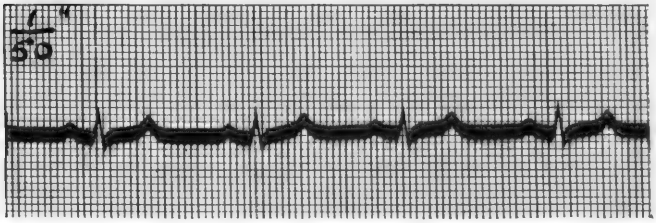

FIG. 15.

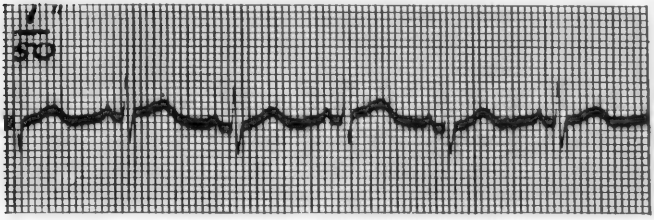

FIG. 16.

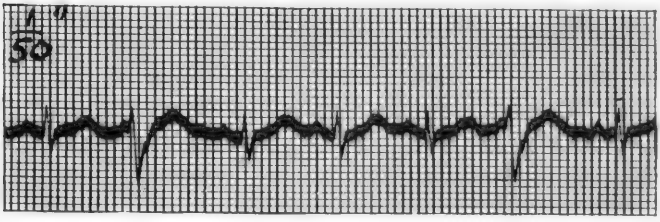

FIG. 17.

(Fraser: Changes in Electrocardiograms.)

PLATE 38.

FIGS. 6 to 9. Rabbit 211 E. Received injections of 0.012 gm. of spartein sulphate per kilo and 0.2 cc. of adrenalin hydrochloride 1 : 1,000 (Parke, Davis and Co.) on Dec. 8, 1913, Jan. 5, Jan. 26, Jan. 30, Mar. 17, Apr. 6, and Apr. 30, 1914.

FIG. 6. Roentgenogram of heart, Dec. 4, 1913.

FIG. 7. Electrocardiogram, Lead II, Feb. 3, 1914.

FIG. 8. Roentgenogram of heart, Apr. 15, 1914.

FIG. 9. Electrocardiogram, Lead II, Apr. 16, 1914.

PLATE 39.

FIGS. 10 to 13. Rabbit 278 E. Received injections of 0.012 gm. spartein sulphate per kilo and 0.2 cc. adrenalin hydrochloride 1 : 1,000 (Parke, Davis and Co.) on Dec. 8, 1913, Jan. 7, Jan. 26, Mar. 17, Apr. 7, and Apr. 30, 1914.

FIG. 10. Roentgenogram of heart, Dec. 4, 1913.

FIG. 11. Electrocardiogram, Lead II, Dec. 2, 1913.

FIG. 12. Roentgenogram of heart, Apr. 6, 1914.

FIG. 13. Electrocardiogram, Lead II, Apr. 7, 1914 (before injection).

PLATE 40.

FIGS. 14 and 15. Rabbit 102 B. Received injection of diphtheria toxin, Jan. 29, 1914.

FIG. 14. Electrocardiogram, Lead II, Jan. 29 (before injection).

FIG. 15. Electrocardiogram, Lead II, Feb. 1, 1914.

FIGS. 16 and 17. Rabbit 118 B. Received injections of suspensions of streptococcus isolated from tonsillar abscess, Apr. 22, May 2, May 18, and May 28, 1914.

FIG. 16. Electrocardiogram, Lead II, Apr. 22, 1914 (before injection).

FIG. 17. Electrocardiogram, Lead II, May 8, 1914.

BACTERIOLOGICAL AND CLINICAL STUDIES OF AN EPIDEMIC OF KOCH–WEEKS BACILLUS CONJUNCTIVITIS ASSOCIATED WITH CELL INCLUSION CONJUNCTIVITIS.[1]

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Plates 41 to 43.

(Received for publication, June 21, 1915.)

While engaged in the study of conjunctivitis associated with epithelial cell inclusions, the occurrence of an epidemic[2] of conjunctivitis due to hemoglobinophilic bacilli enabled us to investigate the relationship between this organism and the epithelial cell inclusions.

Since the discovery of the inclusions by von Prowazek and Halberstaedter, who considered them to be the etiological agents of trachoma, an abundant literature has arisen. Opinions are still greatly divided as to the nature of the bodies, although the majority of investigators regard them as an independent organism, as claimed by von Prowazek and Halberstaedter. They do not, however, commit themselves to the statement as to whether the organism represents the causative agent of trachoma or not. We were inclined to the belief that it constitutes an independent organism which produces a true conjunctivitis of a more or less characteristic clinical course. Herzog[3] considered them to be mutation forms of the gonococcus, while Williams[4] views them as the cell inclusions of various organisms, such as the hemoglobinophilic bacillus and allied species.

[1] Read before the Ophthalmological Section of the New York Academy of Medicine, May 17, 1915.

[2] Service of Dr. Martin Cohen at the Randall's Island Hospital, New York.

[3] Herzog, H., *Arch. f. Ophth.*, 1910, lxxiv, 520.

[4] Williams, A. W., *Collected Studies from the Bureau of Laboratories, Department of Health, City of New York*, 1912–13, vii, 159–247; *Jour. Infect. Dis.*, 1914, xiv, 261.

Although by means of a special method an organism strikingly similar to the epithelial cell inclusions has been made to grow in pure culture,[5] their pathogenic properties, nevertheless, have not yet been established.

As a result of our previous studies a tentative conclusion had been reached that infection of the conjunctiva with these organisms produces an independent conjunctival disease which may properly be described as cell inclusion conjunctivitis. This organism may be present alone, or it may be associated with other pathogenic organisms.

Outbreak and Course of the Present Epidemic of Koch-Weeks Bacillus Conjunctivitis.

Prior to the outbreak of the Koch-Weeks bacillus epidemic,[6] one of us (Cohen) had under observation for about five months 10 cases of inclusion conjunctivitis and 4 cases at the outdoor department of the Post-Graduate Hospital. In these cases, smears (and in a few cases, cultures) were taken weekly and examined (Noguchi), but no Koch-Weeks bacilli were found. On October 3, 1914, one of the inclusion cases under observation at the Island developed an acute conjunctival inflammation. In the conjunctival smears numerous Koch-Weeks bacilli, as well as epithelial inclusions, could be demonstrated. The acute conjunctival inflammation spread to the remaining nine inclusion cases. The smears from all showed inclusions as well as numerous Koch-Weeks bacilli.

In an adjoining ward there were under treatment 15 cases of follicular conjunctivitis, 3 cases of interstitial keratitis, and 1 case of chronic dacryocystitis. 13 of these contracted the Koch-Weeks bacillus infection. During the entire course, which lasted from two weeks to seven months, no pathogenic organisms but the Koch-Weeks bacilli could be demonstrated in the smears and cultures.

There were 7 patients in the trachoma ward. 2 of these showed simultaneous presence in the conjunctiva of inclusion and of Koch-

[5] Noguchi, H., and Cohen, M., *Arch. of Ophth.*, 1914, xliii, 117; *Jour. Exper. Med.*, 1913, xviii, 572.

[6] In the present article the hemoglobinophilic bacilli found in these cases were designated as Koch-Weeks bacilli, in order to conform with the usage of this term in ophthalmology and bacteriology when speaking of this class of organisms.

Weeks bacilli. The latter organisms were present only in cultures, as there were possibly too few to be recognized in the smears. This condition was present for a short time during this epidemic. The infection with the Koch-Weeks bacillus apparently produced no change in the clinical picture of these trachoma cases. The 4 inclusion cases not treated on the Island showed at no time during the past year the Koch-Weeks bacillus, either in smears or cultures; not even during a relapse or reinfection, of which one of these cases had two, could they be demonstrated. Table I shows the bacteriological findings in the cases on which the present report is based.

TABLE I.

Organisms present.	No. of cases.
1. Epithelial cell inclusions alone	6
2. Epithelial cell inclusions at first, with subsequent Koch-Weeks infection	17
3. Koch-Weeks bacillus alone	13
4. Koch-Weeks bacillus at first, with subsequent appearance of inclusions	2
5. Pneumococcus with inclusions	1
Total	39

In acute catarrhal conjunctivitis it is at times difficult, or impossible, to determine the causative organism from the clinical manifestations alone. This is due to the great variability in virulence of the usual infecting organisms, and to the resisting qualities of the individual patient. Yet in many of the acute infections of the conjunctiva, the etiological diagnosis as made by the clinical appearance could be confirmed subsequently by the bacteriological diagnosis, especially in the subacute state. In mixed infections, such a diagnosis is much more difficult.

In a few instances, even smears and cultures of the conjunctival scrapings fail to clear up the diagnosis, as no pathogenic organisms can be found. That the microscope alone is not sufficient to exclude the presence of an organism is shown by the fact that the scrapings from cases of trachoma which showed neither inclusions nor the Koch-Weeks bacilli (as proved similarly by cultures), when inoculated on the conjunctiva of the higher apes (baboons) produced symptoms similar to those which are seen in inclusion cases in man. They were, however, of a milder type and lasted only about ten days. The conjunctival scrapings from the inoculated animals re-

vealed the presence of epithelial inclusions, but no Koch-Weeks bacilli or allied organisms appeared in the smears or in the cultures.

We shall now discuss the clinical manifestations in the group of cases mentioned in Table I.

The clinical course of inclusion conjunctivitis has already been described by Cohen.[7] In the early stage, the clinical manifestations resemble those seen in the Koch-Weeks bacillus infection, and it is only by the examination of smears or cultures that the diagnosis can be positively made. Briefly, they are as follows: In the beginning there is moderate edema of the lids, with mucopurulent secretion. Somewhat later isolated reddish translucent follicles appear in the lower palpebral conjunctiva and upper folds, these two sites becoming involved simultaneously. At a later stage, the upper palpebral conjunctiva takes on a brick red color and assumes the characteristic granular appearance. These regular and progressive manifestations of the disease retrogress after about two months by absorption of the contents of the follicles and papules. These disappear first from the upper portions of the conjunctiva, when the conditions resemble those of cases of follicular conjunctivitis; and later, in about three months, they begin to disappear from the lower conjunctiva. At the end of four months, the conjunctiva is again in a normal condition.

This is the usual course, but at times there are deviations, due to external or unknown conditions, or to a previous affection of the conjunctiva; as, when grafted on a follicular conjunctivitis, the original follicles become larger and new ones make their appearance. In the stage of retrogression the papules become absorbed and the original follicles, owing to relaxation of surrounding pressure, again become prominent, the course being then one of a follicular conjunctivitis. In 2 cases of inclusion conjunctivitis, there remains solely, at the end of nine months, a fine papillary condition of the upper tarsal conjunctiva; one of these two cases has had two relapses or infections.

It may be mentioned here in passing, that of the 75 cases of inclusion conjunctivitis studied by us during the past five years, 58 cases are still under observation of Dr. Cohen. 2 of these still show a diffuse and linear cicatrization of practically the entire palpebral conjunctiva, but no corneal or other involvement is present. 2 other cases of this group still show a fine papillary hypertrophy of both tarsal conjunctivæ, with no corneal or other complications. The remaining 54 cases have remained normal for the past four and a half years. 17 cases were observed for six months, and were normal when last seen.

4 patients with follicular conjunctivitis became infected with inclusions. The follicles previously present became enlarged and congested, and new papules and follicles appeared on the upper tarsal conjunctiva and the conjunctiva of the lower lid. These cases had the appearance of a severe type of inclusion conjunctivitis.

For several weeks after the beginning of the disease, the smears in all cases showed an abundance of epithelial cell inclusions. Toward the end of the disease, the number of inclusions gradually diminished. Yet after three months' duration, although no inclusions were demonstrable in the smears, the clinical aspect of the disease was usually still evident.

[7] Cohen, M., *Arch. of Ophth.*, 1913, xlii, 29.

On Oct. 23, 1914, one of the inclusion cases on the Island developed a slight conjunctival inflammation, which smears and cultures showed to be due to the Koch-Weeks bacillus. In the succeeding two weeks, as previously mentioned, additional cases of inclusion conjunctivitis contracted the Koch-Weeks bacillus infection. Conjunctival smears and cultures examined weekly for the past several months showed at first the simultaneous presence of inclusion and Koch-Weeks bacilli. In the succeeding two months, the findings varied, at times only one of the two organisms being present, at other times, both. Possibly this was due to relapse or reinfection. In the next two months, although no organism was present in smears and cultures, there was still evidence of inflammation. Ultimately the conjunctiva became normal in all but one case. In this case there is still at the end of a year evidence of an inclusion conjunctivitis in the retrogressive stage.

In an adjoining ward, where mild follicular conjunctivitis cases were being treated, 13 contracted the Koch-Weeks bacillus infection. Previous to the Koch-Weeks infection, all these cases showed over various areas of the conjunctiva follicles varying in number and size, and showing no inflammatory reaction. The first case in this ward developed on Nov. 1, 1914. Only 3 of the 16 cases escaped the Koch-Weeks infection. The Koch-Weeks bacillus was present in great numbers, but no other pathogenic organisms could be demonstrated throughout the entire course of the epidemic, which lasted six months.

The clinical manifestations in these follicular cases were as follows. There were slight edema of the lids and conjunctival congestion with mucopurulent secretion. Both eyes became successively involved. The acute infection lasted six to eight weeks. At the end of this period, the original follicles were still present.

In one of the typical follicular cases it was difficult to determine whether the acute infection was due to the Koch-Weeks bacillus or to the epithelial inclusions. The symptoms were those of a severe inclusion conjunctivitis. But notwithstanding repeated examinations extending over two months, no inclusions could be found. Nevertheless, judging from the clinical course and from the fact that cell inclusions are often absent in the subacute stage, this and similar cases may be considered due to epithelial inclusions.

2 cases of interstitial keratitis on the Island Eye Ward and 1 at the Dispensary had normal conjunctivæ, when they suddenly developed an acute catarrhal conjunctivitis. In the smears and cultures, which were taken weekly for four months, the Koch-Weeks bacillus was present, even at a time when the conjunctiva was free from inflammation. The inflammatory stage lasted from six to eight weeks. Inclusions could at no time be demonstrated in the smears.

The clinical appearance in these cases was moderate edema of the lids, and conjunctival congestion with mucopurulent secretion. In one case there was present a small scleroconjunctival hemorrhage with a small phlyctena. Conjunctival furrows or folds also appeared in those cases when the congestion diminished.

As regards the last group in the table, 2 cases at first showed the Koch-Weeks bacillus in the conjunctival smears. Later, inclusion cells made their appearance, associated with their clinical manifestations. In the first case there was present

a mild catarrhal conjunctivitis, papules being present on the lower tarsal conjunctiva alone. In this respect it differed from other inclusion cases where the papules are present also on the upper tarsal conjunctiva. It is possible that the lesions at times can only be detected microscopically. A second examination in this case revealed the epithelial inclusions. Perhaps the inclusions were missed in making the first examination, as it is likely that in some stages of the disease only a few epithelial cells contain inclusions.

The second patient originally had a follicular conjunctivitis. He then contracted an acute catarrhal conjunctivitis, the secretion containing for three weeks the Koch-Weeks bacillus alone. At the end of this time, when the conjunctivitis improved, he again developed an acute conjunctival inflammation, and the smears showed numerous inclusions and a few Koch-Weeks bacilli. This case then followed the usual course of an inclusion conjunctivitis, the conjunctiva ultimately returning to a normal condition.

In a ward assigned to minor eye affections, where there were a few mild Koch-Weeks cases, a routine examination of the conjunctivæ of all the cases was made, in order to determine the presence of the Koch-Weeks bacillus. 2 cases showed the Koch-Weeks bacilli, in spite of the fact that the conjunctivæ were entirely normal throughout the whole period. In another case in the same ward degenerated Koch-Weeks bacilli were found in the conjunctival smears. This case then developed an acute inflammation, showing all the symptoms of an inclusion conjunctivitis with the added findings of epithelial cell inclusions in the smear examination.

In our routine examinations made during the past five years Koch-Weeks bacilli have rarely been found. Recently we encountered eight cases of pneumococcal conjunctivitis. Clinically these cases resembled acute catarrhal conjunctivitis, due to the Koch-Weeks bacillus, and were so diagnosed before the smears and cultures showed the presence of numerous pneumococci without any Koch-Weeks bacillus. The conjunctival secretions in these cases were typically serous, being also associated with diffuse congestion. The duration was from one to two weeks. One of the patients, included already in this paper, had originally an inclusion conjunctivitis. Later, he contracted a Koch-Weeks bacillus infection. After four weeks, when apparently cured, another acute inflammation appeared, which was due to the pneumococcus. This infection was followed by the appearance of inclusion conjunctivitis, the smears showing both pneumococcus and inclusion cells. At present, the clinical symptoms are those of an inclusion conjunctivitis in a state of relapse or reinfection.

The treatment adopted in all these cases was irrigation of the conjunctival sac with a saturated solution of boric acid every two hours, and the application to the conjunctiva, once a day, of a silver nitrate solution, until the acute symptoms had subsided, when irrigation alone was continued. This method of treatment had practically but little effect on the course of the inclusion cases, whereas in the Koch-Weeks infections decided improvement was observable. The disappearance of the organisms from the smears and culture was, however, not at all influenced by the application of the silver nitrate.

Bacteriological and Experimental Studies of the Present Epidemic.[8]

Our technique for obtaining smears and cultures was as follows. The upper lid was everted and then the short end of a sterile slide was gently rubbed over the upper tarsal conjunctiva where there is least likelihood of contamination. By means of a platinum loop, a part of the scrapings removed was used for the purpose of cultivation and animal experiments. The remainder of the materials on the slide was transferred to another slide where a thin spread was made, and examined by means of the Giemsa or Gram stain.

Since our problem deals with the Koch-Weeks infection, the culture media employed were chiefly blood agar and tissue ascitic fluid. The cultural findings are summarized below.

In the present epidemic it was noticed that various strains of the so called Koch-Weeks bacilli or hemoglobinophilic organisms in cultures varied in size to such an extent that they may be divided into a thin (Figs. 4 to 7), a medium (Figs. 8 to 11, 20 to 23), and a coarse type (Figs. 16 to 19), according to their morphology. The coarse variety were bacillary or coccoid (Figs. 12 to 15) and resembled culturally and morphologically some strains of *Bacillus influenzæ* (Figs. 24 and 25) derived from the respiratory organs; while the thinner variety was much narrower and often shorter. After several days' cultivation at 37° C. on blood agar, some of the organisms became somewhat granular and unevenly stainable; when found in large masses they often assumed the appearance of the granular forms of the so called trachoma bodies (Figs. 5 to 7, 9 to 11, 13 to 15, 17 to 19). But these granules did not break down so as to approach the minuteness of the elementary bodies (Figs. 32, 33, 36, 39, 43, 45) which are found in the uncomplicated cases of inclusion conjunctivitis or trachoma. The degenerated or involuted bacilli do not have the sharp contour of the elementary granules. Transplants made from these somewhat degenerated granular masses of the hemoglobinophilic organisms give a good growth of typical bacillary or semicoccoid forms. The character of the colonies on the blood agar is also distinctive, in that they remain minute, sharply elevated with a pointed top, and appear more grayish than the colonies of the

[8]This part of the work was carried out at The Rockefeller Institute.

influenza type varieties, which show a tendency to spread and are of a more dewy aspect. It is quite possible that the minute type constitutes a group by itself, but further study is required to determine its relation to the other groups. At all events several strains of influenza bacillus[9] derived from cases of meningitis and pneumonia appear quite different from the conjunctivitis strains in their morphological features (Figs. 24 to 28). The frequency with which organisms of different type were found in the present epidemic is shown in Table II.

TABLE II.

Organisms present.	Types.		
	Coarse.	Medium.	Thin.
1. Epithelial inclusions first, with subsequent Koch-Weeks infection (17 cases)	1	7	9
2. Koch-Weeks bacillus alone (13 cases)	1	10	2
3. Koch-Weeks bacillus first, with subsequent appearance of inclusions (2 cases)	1 (round type)	1	

As will be seen from Table II, the thin variety occurred very frequently in the cases in which the patients had been previously infected with the inclusions. In the simple Koch-Weeks cases the medium was predominating. It is significant that in the smears taken from the cases infected with the inclusions and Koch-Weeks bacilli it is not difficult to differentiate the initial bodies of the former from the bacilli, because the initial bodies are much larger and oval in shape, and take up a deeper blue stain. Fig. 3 shows a typical inclusion near the nucleus and some Koch-Weeks bacilli in the same cell. The hemoglobinophilic bacilli are seen to be taken up by the polynuclear leucocytes more frequently than by the epithelial cells (Fig. 1), while the inclusion bodies are chiefly epithelial and seldom leucocytic in nature (Figs. 29 to 45). So far as could be ascertained in stained specimens the bacilli remain well preserved within the cells and do not seem to disintegrate into granules (Fig. 2). On the other hand, the initial bodies are often ill defined and do not show any distinct bacillary forms. They are far more

[9] We are indebted to Dr. Martha Wollstein for these strains, for which we here wish to express our thanks.

pleomorphic than the hemoglobinophilic bacilli (Figs. 29, 34, 37, 40, 41, 43, 46, and 47).

The isolation of the hemoglobinophilic organisms, irrespective of the type, was easily accomplished by means of the blood agar; but in many instances the preliminary microscopical search in the smears failed to demonstrate the presence of a few organisms, until they could be found by the cultural procedure. It must be understood that in cases where the inclusions only were found, repeated efforts to isolate the hemoglobinophilic organisms were made, with, however, invariably negative results. In this connection it may be added that a number of cases clinically diagnosed as trachoma was also studied in order to see if any hemoglobinophilic organism could be isolated. The results were uniformly negative. A few cases were also found in the same wards, which may have been infected through contact with the inclusion cases and which, although carefully observed from the earliest stage of the disease, did not at any period show the presence of any hemoglobinophilic bacilli.

Transmission of the Inclusion Bodies to Animals.—In order to find out whether the inclusion virus ever exists in the form of a hemoglobinophilic bacillus, scrapings from the conjunctivæ of these cases were inoculated into the conjunctiva of a baboon. Prior to the inoculation the conjunctiva of the baboon was examined to ensure the absence of such a bacillus. Within seventy-two hours after the inoculation the conjunctiva showed moderate congestion, edema, and a few minute papules; a small quantity of a mucopurulent discharge was present at the inner canthus. The smears and culture were made from the conjunctival scrapings. The result showed that there were numerous cell inclusions, but no hemoglobinophilic organisms[10] (Figs. 46 to 51). The examinations were continued regularly for a period of two weeks and the results were unvarying. As was previously shown by various investigators, the von Prowazek bodies are transmissible to higher apes, but the hemoglobinophilic or Koch-Weeks bacillus is not.

Attempts To Produce Koch-Weeks Bacillus Conjunctivitis in Animals.—Several attempts were made to transfer the hemoglobino-

[10] Cultures made after 24 and 48 hours were also negative in regard to the hemoglobinophilic bacillus.

philic organisms to the conjunctivæ of rabbits (young and adult) and monkeys (baboon and several *Macacus rhesus*), by introducing several loopfuls of the twenty-four hour blood agar cultures of different strains into moderately abraded surfaces of conjunctivæ. The results were completely negative, except in the case of one rabbit, where the organism was still recoverable after twenty-four hours. It seems remarkable that such a large quantity of pure cultures of freshly isolated strains of these organisms from the cases where the inclusions were also present should fail to reproduce the conjunctivitis, in view of the fact that a comparatively small number of the inclusions as contained in the scrapings from a patient can readily reproduce the inclusion conjunctivitis.

From this a conclusion may be warranted that in a conjunctivitis where the inclusion and the hemoglobinophilic bacilli are simultaneously present, two pathogenic factors can be separated by means of transmission of the material into the conjunctiva of a suitable animal (baboon) in which the inclusion virus alone implants itself upon the new host, while the bacilli quickly disappear from the inoculated conjunctiva. For man both organisms are pathogenic, but for the baboon only the inclusion virus is capable of producing infection.

On the other hand, this may not exclude the possibility, as asserted by Williams and her associates, that a conjunctivitis due to the Koch-Weeks bacillus may also show some cell inclusions; since under certain experimental conditions a very suggestive phenomenon, to be related below, has been observed.

Attempts To Produce the Koch-Weeks Bacillus Epithelial Inclusions in Animals.—In order to determine experimentally whether or not the hemoglobinophilic bacilli when taken up by epithelial cells will undergo the morphological changes which lead to the formation of so called inclusion bodies, an intratesticular inoculation of the rabbit with pure cultures of the hemoglobinophilic organisms isolated from the cases already mentioned was resorted to. The local reaction which follows consists of edema of the scrotum and induration of the testicular parenchyma within twenty-four hours. The edema gradually disappears within the next few days, while the testicular induration remains more or less the same for about five days, after which it commences to recede. In some instances the

rabbit succumbed to septicemia and probably to intoxication as a result of the introduction of the cultures. The organs were removed at intervals of twenty-four hours, three days, and six days, and then fixed in sublimate alcohol, and stained by Giemsa's acetone method,[11] and, if overstained, treated with a 10 per cent solution of *Glycerinäthermischung* (Grübler) for a few minutes, as advocated by one of us.[12] The results show that the injection of the bacilli is followed by an intense leucocytosis, in which the polynuclears invade the tubules in groups. In the twenty-four hour specimens the organisms are still well distributed along the interstitial spaces; in the three day specimens one notices numerous masses of agglutinated bacilli here and there within the tubular lumina or along the connective tissue. These masses take on a purplish hue and appear granular in structure and indefinite in outline. They are on the point of disintegration. The granules within and about these bacterial masses are not so minute as to be mistaken for the elementary bodies of the inclusion. In the six day specimens some clumped bacteria were found within the polynuclear leucocytes, but a diligent search failed to show any typical epithelial cell inclusions. In these six day preparations the number of the bacterial clumps is smaller than at an earlier period. There were no granules small enough to be regarded as the typical elementary granules. While a careful comparison of the inclusions and the bacterial clumps just referred to will reveal the difference between them, this is not always easy to accomplish (Figs. 53 to 55). Fig. 52 shows a mass of granules from a case of mixed infection of Koch-Weeks bacilli and the von Prowazek inclusions, and it appears difficult to determine whether they represent the degenerated bacilli or the inclusion granules. The deep stain of the mass and the absence of any free bacilli around it seem to indicate that it belongs to the latter kind. It is also possible that some of the clumped granules found in the conjunctival smears from cases of Koch-Weeks infection might have been interpreted as the cell inclusions and classified with the von Prowazek inclusions.

Attempts To Transmit the Inclusion Bodies to a Parenchymatous Organ in Animals.—Efforts were also made to transmit the von

[11] Giemsa, G., *Deutsch. med. Wchnschr.*, 1909, xxxv, 1752.

[12] Noguchi and Cohen, *Proc. N. Y. Path. Soc.*, 1910, x, 20.

Prowazek bodies from uncomplicated inclusion cases to the testicles of rabbits, since this organ offers an excellent medium of growth to various highly parasitic organisms which otherwise cannot be easily cultivated.[13] The scrapings of conjunctivæ from 4 different patients were inoculated into the testicles of 8 rabbits; but in spite of the large number of the inclusions contained in the conjunctival scrapings used for this purpose, no success was obtained along this line. The testicles showed within twenty-four hours some induration and edema, but after a few days resumed their normal condition. Tissues removed after twenty-four hours, three days, and six days failed to show any cell inclusions when examined in smears and sections. No hemoglobinophilic organism was found in cultures made from the tissues. This negative finding also tends to strengthen the view that the cell inclusions found in these cases were not the Koch-Weeks bacilli, for if they had been it would have resulted in the production of Koch-Weeks orchitis.

CONCLUSIONS.

1. There are cases in which epithelial cell inclusions may alone be present in the conjunctival smears. In such cases no other pathogenic organisms, such as the Koch-Weeks bacillus or the pneumococcus, can be demonstrated in smears or cultures.

2. The conjunctiva can become simultaneously infected with the inclusion bodies and Koch-Weeks bacilli or other organisms.

3. In cases of acute or subacute conjunctival inflammations due to mixed infections the clinical features of each infection may be present. The course of the inflammation is, however, more prolonged.

4. Within recent years, the Koch-Weeks bacillus has only seldom been found in our routine examinations.

5. The epidemic studied was of a severe type.

6. Clinically it is practically impossible to distinguish pneumococcal conjunctivitis from the Koch-Weeks conjunctivitis. Bacteriological examination of smears and cultures is the only means by which the etiological diagnosis can be definitely established.

7. Conjunctivæ of certain species of monkeys are susceptible to

[13] Noguchi, H., *Jour. Exper. Med.*, 1915, xxi, 539.

the von Prowazek inclusion bodies, but not to the hemoglobinophilic bacilli isolated from cases of epidemic conjunctivitis.

8. The injection of conjunctival scrapings containing the von Prowazek cell inclusions into the testicles of rabbits produces no cell inclusions in the latter, while the injection of a pure culture of the hemoglobinophilic bacilli causes an acute inflammation accompanied by numerous clumps of the organisms, simulating the von Prowazek bodies at certain stages of their evolution.

9. There exists an apparent morphological similarity between the degenerated forms of this variety of the hemoglobinophilic bacilli and the cell inclusions, both in cultures and in experimental orchitis in the rabbit. But, as a rule, the elementary bodies of the latter are much smaller and more sharply defined than the smallest granules of the former, while the initial bodies are bigger, more intensely stainable, and less definite in their contour than the hemoglobinophilic bacilli found in the infected conjunctivæ.

EXPLANATION OF PLATES.

All the photographs were made from film preparations, except where otherwise stated. They were stained with Giemsa. The enlargement is uniformly 1,000 diameters.

PLATE 41.

FIG. 1. This shows a mass of the Koch-Weeks bacilli in a film preparation from an uncomplicated case of Koch-Weeks conjunctivitis. The bacilli belong to the thin type and some scattered examples appear coccoid. By focusing, the mass is seen to be composed of numerous well defined bacilli which cannot be confused with the inclusion granules.

FIG. 2. An epithelial cell from a case of uncomplicated Koch-Weeks conjunctivitis. Around the periphery of the cell and near the nucleus along the lower border numerous bacilli are seen to be attached to, or contained within, the cell body. There is, however, no difficulty in recognizing the bacilli as such in this instance.

FIG. 3. A film preparation from a case of mixed infection of the Koch-Weeks and the inclusion organisms. In the field the epithelial cell is seen to contain a densely stained mass of the initial bodies near the upper right border of the nucleus and numerous Koch-Weeks bacilli to the left, especially where there is a leucocyte.

FIG. 4. This represents a twenty-four hour old pure culture of a thin type of the Koch-Weeks bacilli on blood agar.

FIGS. 5, 6, and 7. The appearance of the same organism as in Fig. 4 after 3 days, 5 days, and 8 days, respectively, on the same medium at 37° C.

FIGS. 8, 9, 10, and 11. These represent the appearance of a medium type strain of the Koch-Weeks bacilli after 24 hours, 3 days, 5 days, and 8 days, respectively, on blood agar at 37° C.

FIGS. 12, 13, 14, and 15. These show the appearance of a strain of round or coccoid type of the Koch-Weeks bacilli in a 24 hour, 3 day, 5 day, and 8 day growth on blood agar, respectively.

FIGS. 16, 17, 18, and 19. These show the appearance of a coarse strain of the Koch-Weeks bacilli in a 24 hour, 3 day, 5 day, and 8 day growth on blood agar, respectively. This strain resembles *B. influenzæ* more than the others.

FIGS. 20, 21, 22, and 23. These show 4 different strains of the Koch-Weeks bacilli isolated from the mixed infection cases in the present epidemic. They belong to the medium type.

FIGS. 24, 25, and 26. These show 24 hour growths of 3 different strains of *B. influenzæ* isolated from cases of pneumonia.

FIGS. 27 and 28. These show 24 hour growths of 2 different strains of *B. influenzæ* isolated from cases of meningitis. Both strains were kept on artificial media for several years and tend to form threads more than they originally did (Wollstein).

PLATE 42.

FIGS. 29, 30, 31, 32, and 33. These show the inclusions at various stages of evolution in the film preparations made from a case (Tho.) of inclusion conjunctivitis. This patient had been suffering from the inclusion conjunctivitis when he was superinfected with the Koch-Weeks bacilli, which, in due course, disappeared from his conjunctiva leaving the original condition little affected. These inclusions shown here were found in the conjunctiva long after the Koch-Weeks bacilli had disappeared in smears or in cultures. The minute elementary bodies in Figs. 32 and 33 bear no resemblance to the degenerated forms of the Koch-Weeks bacilli.

FIGS. 34, 35, and 36. These represent the inclusion bodies in an uncomplicated case (Sh.), that is, without any association with the Koch-Weeks bacilli. The character of the initial bodies in Fig. 34 would definitely dispose of any suggestion as to their being the Koch-Weeks bacilli. Fig. 36 shows a still unburst intracellular aggregation of the elementary bodies.

FIGS. 37, 38, and 39. Inclusions from another case (Sat.) of pure inclusion conjunctivitis.

FIGS. 40, 41, and 42. Inclusions from a case (Bol.) of trachoma. The initial bodies as shown in Fig. 40 are exceptionally coarse.

FIGS. 43, 44, and 45. Inclusions from an uncomplicated case (For.) of inclusion conjunctivitis. In Fig. 43 a thick mass of the initial bodies is seen to embrace the left side of the nucleus of a cell on the left, while on the right numerous elementary granules are scattered around the cell below. Figs. 44 and 45 show the steps of evolution of the inclusion bodies.

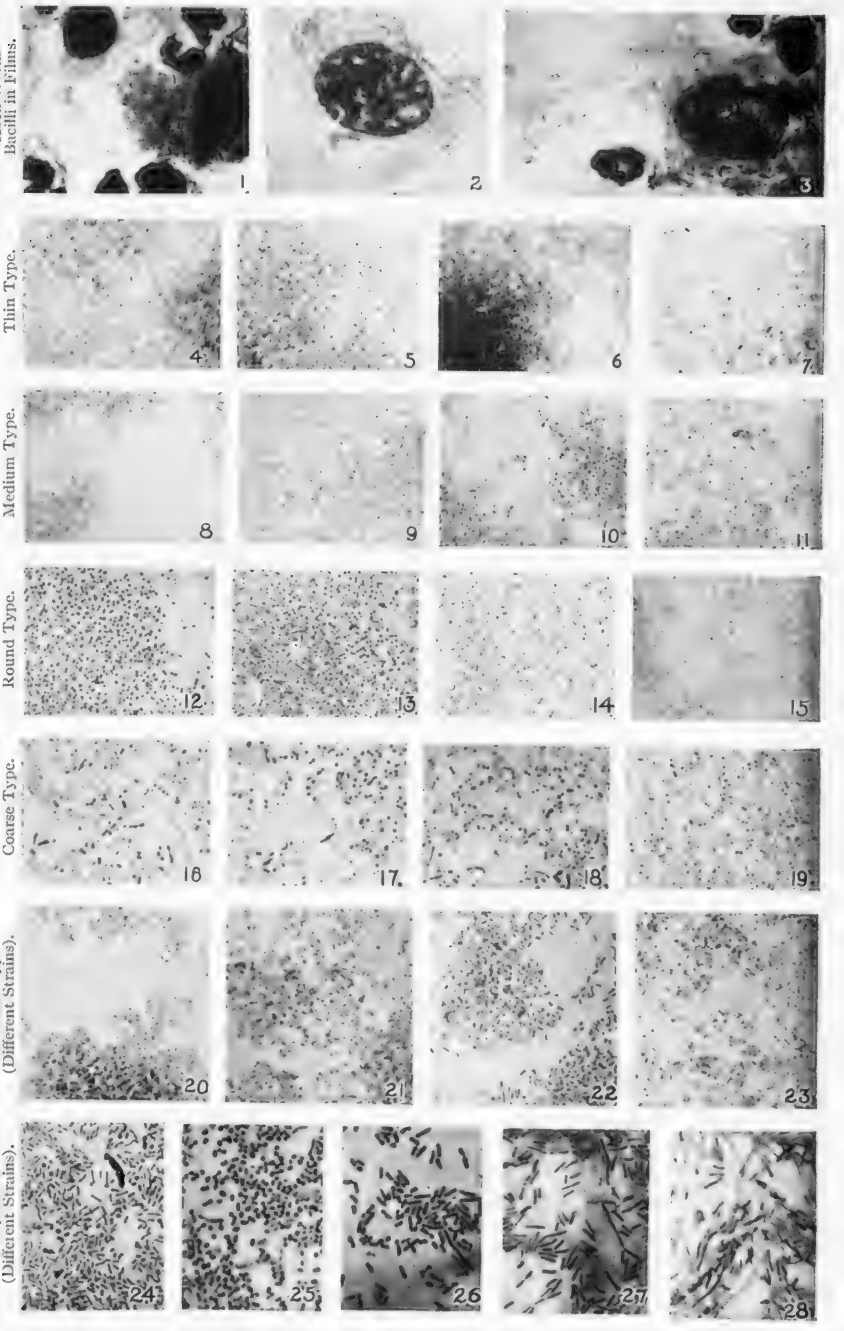
PLATE 43.

FIGS. 46, 47, 48, 49, 50, and 51. Different stages of the evolution of the inclusion bodies and the conjunctiva of a baboon experimentally infected with the scrapings of the affected conjunctivæ of man. The structure of the initial

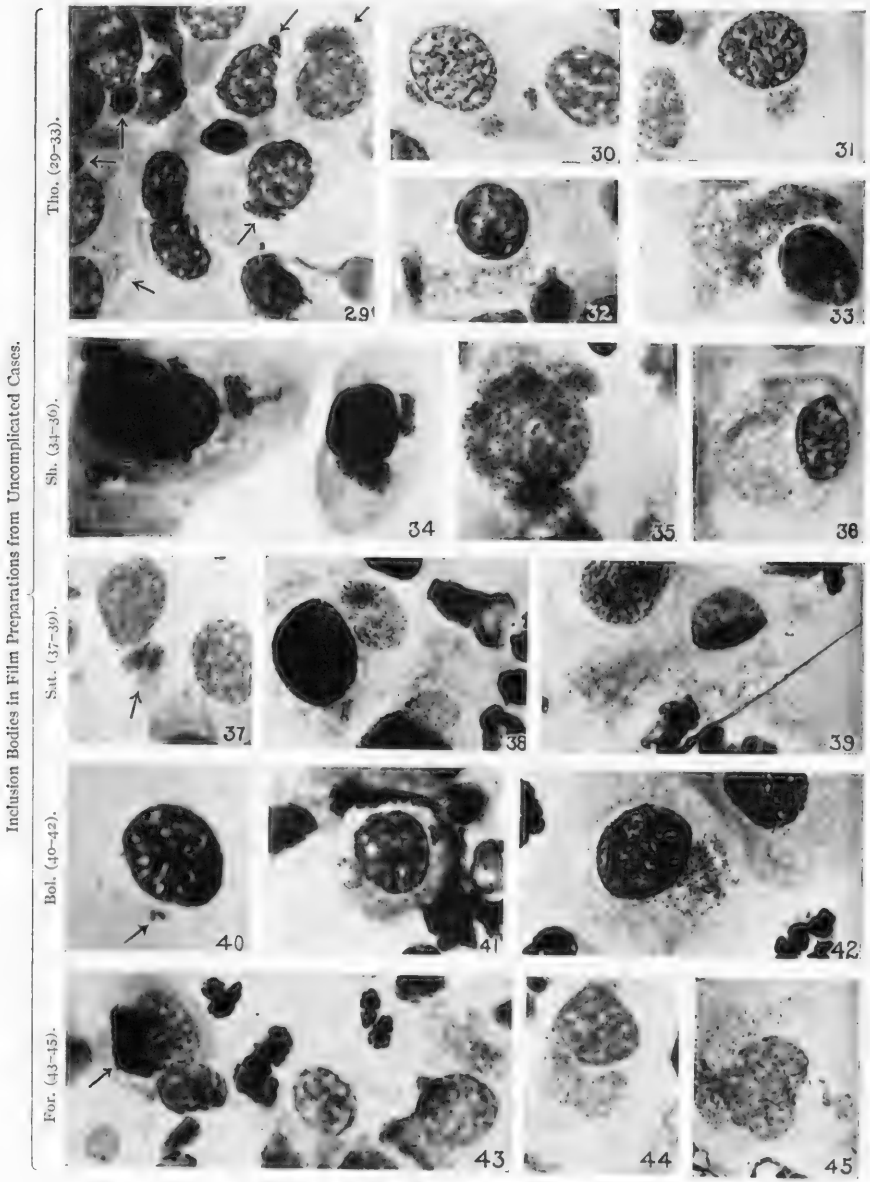
bodies in Figs. 46, 47, and 48 is quite peculiar and reveals no definite bacillary outlines. They are deeply stained, almost amorphous, and show indistinct coarse granules within the mass. The elementary bodies are even more minute than those in the human cases, as will be seen in the cell occupying the lower half of Fig. 48. In the same figure one sees an irregularly shaped mass of the initial bodies at the upper corner. In Figs. 49, 50, and 51 are shown the enormously distended cells in which the inclusion granules are rapidly multiplying.

FIG. 52. This shows a dense mass of deeply stained granules in a film preparation from a case of mixed infection. This mass appears to be more of the von Prowazek type of body than of the degenerated Koch-Weeks bacilli.

FIGS. 53, 54, and 55. Sections of the rabbits' testicles inoculated with pure cultures of Koch-Weeks bacilli isolated from the mixed infection cases during the present epidemic. Fig. 53 shows that the organisms assume a coccobacillary form when examined within twenty-four hours after they were injected into the testicle. In photography they appear somewhat like the initial bodies in the inclusion cases, yet in actual examination of the preparations they are not difficult to distinguish as the Koch-Weeks bacilli. They are freely capable of cultivation when a minute portion of the testicle is transferred to the blood agar, while this is not the case with the initial bodies of the inclusion organism. In Figs. 54 and 55 the sections removed after 3 and 6 days, respectively, are shown in which large masses of agglutinated and somewhat ill defined bacilli are found in clear spaces within the lumen of the testicular tubules. They, too, present an appearance not unlike that of the inclusion organism found in experimental conjunctivitis in monkeys, as well as in an epidemic in man. Here, again, a culture on blood agar is quickly differentiated from the other kind, as the Koch-Weeks bacilli at this stage still thrive very well in culture, while the pure inclusion material does not give any growth with the hemoglobinophilic bacilli.

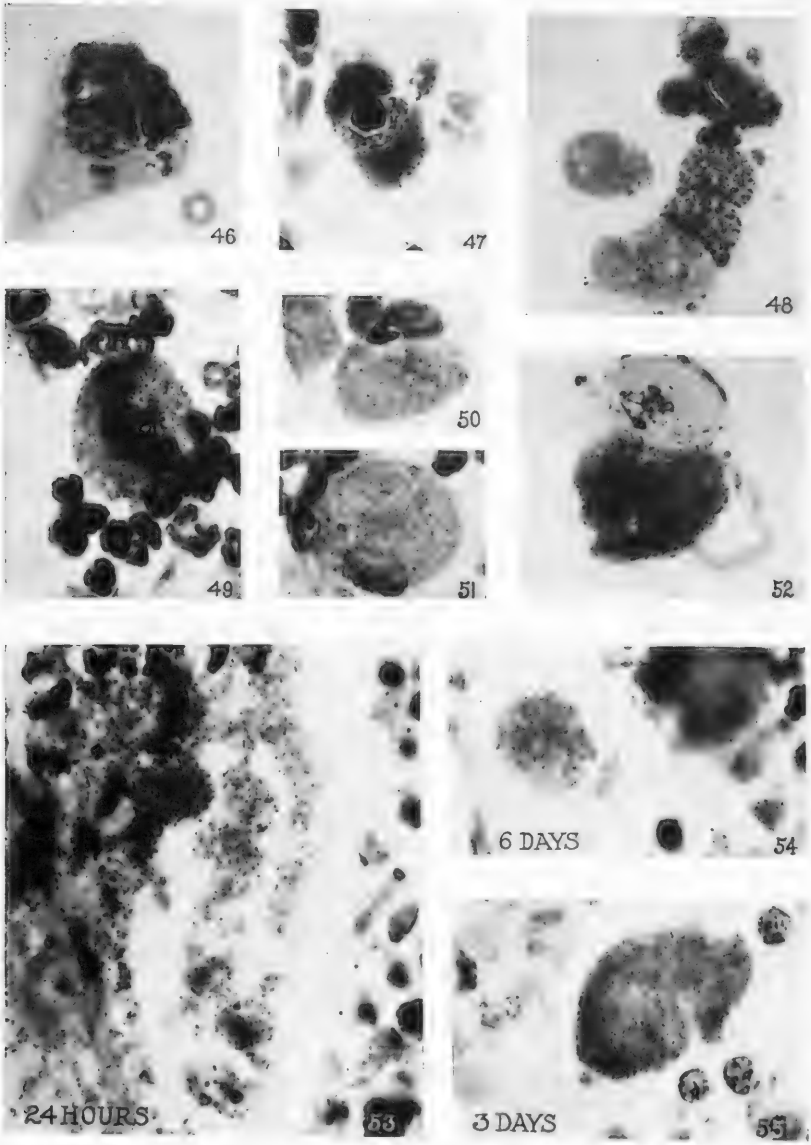


(Noguchi and Cohen: Epidemic of Koch-Weeks Bacillus Conjunctivitis.)



(Noguchi and Cohen: Epidemic of Koch-Weeks Bacillus Conjunctivitis.)

Experimental Inclusion Conjunctivitis in Baboon (46–51 .



Experimental Koch-Weeks Orchitis in Rabbit (53–55).

(Noguchi and Cohen: Epidemic of Koch-Weeks Bacillus Conjunctivitis.)

STUDIES OF METABOLISM IN THE DOG BEFORE AND AFTER REMOVAL OF THE SPLEEN.

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(Received for publication, June 4, 1915.)

In contradistinction to the uniformly negative results obtained by those who have studied the influence of the spleen on metabolism are the observations of Richet (1), which seem to indicate that in order to maintain the weight of a splenectomized dog a much larger amount of food is required than is the case with a normal dog. Richet's studies suggested to us a possible explanation of certain contradictory and confusing results which have been obtained in this laboratory during a lengthy study of the influence of diet upon the anemia which follows removal of the spleen. Unfortunately, however, Richet investigated only the weight of the animals in relation to the amount of food given and did not attempt a general study of metabolism. Moreover, a study of the literature of this subject shows that although a very complete metabolism study has been made by Paton (2), who decided that the spleen has no importance in metabolism, no one has made metabolic studies after splenectomy in animals in a state of nitrogen equilibrium. We therefore undertook the present investigation in the hope of throwing some light upon the dietary studies to which we have referred, and also, if possible, to clear up the apparent discrepancy between the results of Richet and of Paton.

Previous Investigations.

Paton's investigation included studies of the nitrogen metabolism and the elimination of salts in a single dog before and after splenectomy. Observations were made during fasting and on (1) meat, (2) oatmeal and milk, and (3) rich nuclein diets. The first postsplenectomy metabolism study was made 26 days and the last 4 months after the operation. Paton's general conclusion is that

under the various conditions of his experiments, splenectomy causes no essential difference in the course or nature of the metabolism.

In Richet's first investigation 9 splenectomized dogs were contrasted with 6 normal dogs. No metabolism studies and no examinations of the blood were made. Conclusions were based on records of food taken and the weight of the animals at various intervals. The increased consumption of food by the splenectomized animals is thought by Richet to be due to an increased catabolism in those animals and not to any disturbance of digestion. In a later report (1) he refers to studies of 17 splenectomized dogs, of which 5 were under observation for about 2 years, and confirms the conclusions of his earlier report. In this connection it is a matter of importance that the conclusions are based on the averages of 2 groups of dogs of widely different weights. Richet has not contrasted splenectomized dogs of given weight with normal dogs of the same weight, but if one selects from his tables dogs of the same weight the differences in food consumption are found to be very slight. Only 2 dogs were studied both before and after splenectomy.

Mendel and Jackson (3) who investigated the relation of the spleen to purine metabolism found that in splenectomized dogs and cats no changes occurred.

Verzár (4) has found that extirpation of the spleen in dogs has no appreciable effect upon the respiratory gas exchange. A similar conclusion was reached by Korenchevski (5) as regards both gaseous and nitrogenous metabolism. No other experimental studies of the influence of splenectomy are available except the brief note of Austin and Ringer (6), to the effect that in the dog the absence of the spleen does not in any way modify the course of the glycosuria caused by phlorhizin.

Metabolism studies in man, before and after removal of the spleen, are few in number, and, moreover, are, in all instances, difficult of interpretation because of the existence of pathological conditions, such as Banti's disease, pernicious anemia, or congenital hemolytic jaundice, on account of the presence of which the organ was removed. As the present study has for its object the determination of the influence of the removal of the normal spleen from a normal animal, the literature dealing with changes following its removal in disease (7) need not be considered at this time.

Methods.

The four dogs used in this study were placed upon a constant diet of beef (usually beef heart), lard, and sugar, the amounts of each of which constituents varied according to the caloric needs of each dog. The standard diet contained 0.4 of a gram of nitrogen per kilo and 70 calories per kilo of body weight. A small amount of sodium chloride was given each day, and a sufficient amount of

bone ash was added to ensure well formed feces. The water intake for each day was constant. To some animals the beef heart was given raw; in other instances it was boiled. After one or two weeks on the special diet, if the weight of the animal remained constant, a preliminary metabolism study, covering a period of seven days, was made. If the results of this were satisfactory the animal was then splenectomized and the metabolism studies were resumed at various intervals after the operation. In each experiment the diet after operation was always the same as before and was continued without change in the intervals between periods of metabolism study. Analyses were made of all foods for fat and total nitrogen. During the periods of study the animals were kept in the usual metabolism cages. They were catheterized at the end of every twenty-four hours and the feces marked by carmine.

In the analysis of the urine the total nitrogen was determined by the Kjeldahl-Gunning method, ammonia by Folin's method (8), creatine and creatinine by Folin's method (9), and the hydrogen ion concentration according to Henderson's technique (10). In the study of the feces the Kjeldahl-Gunning method was used for total nitrogen, the Folin-Wentworth method for fat (11), and Neumann's method (12) for iron.

The removal of the spleen, an essentially bloodless operation, was done under ether anesthesia. These operations were performed by Dr. Max M. Peet, of the Department of Surgery.

RESULTS.

The details of our studies of nitrogen metabolism are shown in Tables I, II, III, and IV; of fat metabolism in Table V; and of the elimination of iron in Table VI.

Nitrogen Metabolism.—Table I shows the earliest period of metabolic study (three days) after splenectomy. The animal shows no loss of weight, no ill effect of the operation, and the conditions were therefore ideal for the detection of any slight changes in metabolism which might be due to the absence of the spleen. No variations in nitrogen partition were observed, however, and the nitrogen equilibrium was maintained: an average daily balance before operation of 0.45 of a gram and after operation of 0.46 of a gram.

TABLE I.

Dog 57. Nitrogen Metabolism before and Three Days after Splenectomy.

Date.	Weight.	N intake.*	Urine.								Feces total N.	N of urine and feces.	N balance.
			Amount.	Specific gravity.	Reaction to litmus.	H ion concentration.**	Total N.	Ammonia N.	Creatinine.	Creatine.			
	kilos	*gm.*	*cc.*	10			*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Feb. 28	10.6	4.80	295	29	Acid	6.90	3.45	0.13	0.289	0.478	0.73	4.18	+0.62
Mar. 1	10.8	4.80	175	34	"	6.50	3.76	0.15	0.270	0.357	0.73	4.09	+0.71
" 2	10.8	4.80	225	37	"	6.90	3.44	0.12	0.289	0.326	0.73	4.17	+0.63
" 3	10.8	4.80	225	38	"	6.90	3.57	0.13	0.285	0.321	0.73	4.30	+0.50
" 4	10.8	4.80	190	37	"	6.90	3.65	0.14	0.289	0.335	0.73	4.38	+0.42
" 5	10.9	4.80	200	40	"	6.90	3.86	0.14	0.289	0.335	0.73	4.59	+0.21
" 6	10.9	4.80	220	27	"	6.90	4.02	0.12	0.279	0.377	0.73	4.75	+0.05
Average..	10.8	4.80	219	35		6.84	3.62	0.13	0.284	0.361	0.73	4.35	+0.45
Mar. 7	Splenectomy.												
" 10	10.8	4.80	150	45	Acid	6.15	3.86	0.18	0.311	0.310	0.53	4.39	+0.41
" 11	10.8	4.80	220	35	"	6.50	3.92	0.15	0.311	0.310	0.53	4.45	+0.35
" 12	10.9	4.80	235	37	"	6.50	3.96	0.14	0.332	0.285	0.53	4.49	+0.31
" 13	10.9	4.80	180	40	"	6.50	4.08	0.13	0.302	0.320	0.53	4.61	+0.19
" 14	10.9	4.80	135	45	"	6.15	3.95	0.15	0.289	0.267	0.53	4.48	+0.32
" 15	10.9	4.80	165	37	"	6.15	3.36	0.13	0.289	0.259	0.53	3.89	+0.91
" 16	11.0	4.80	210	42	"	6.90	3.53	0.08	0.311	0.283	0.53	4.06	+0.74
Average..	10.9	4.80	185	40		6.41	3.81	0.16	0.306	0.291	0.53	4.34	+0.46

* Diet: raw beef, 150 gm.; lard, 50 gm.; sugar, 50 gm.

** Expressed as negative logarithms.

Table II shows practically the same results, 13 days and 8 weeks after splenectomy. The animal was in nitrogen equilibrium before splenectomy and maintained that condition after splenectomy. The general metabolism shows entirely normal results. The utilization of nitrogen was in no way interfered with; it was 94 per cent before operation and 95 and 93 per cent in the postsplenectomy periods.

In Table III, which presents observations 2, 6, and 10 weeks after splenectomy, the results in the third and fourth periods (sixth and tenth weeks) are similar to those shown in Tables I and II. In the early period after splenectomy, however, this animal showed a loss of appetite which caused, during the two weeks following operation, a loss in weight of 1.4 kilos. This loss of appetite was not due to infection or other postoperative disturbances, but appeared to be due rather to a dislike of the lard in the diet. When

TABLE II.

Dog 48. Nitrogen Metabolism before and Two and Eight Weeks after Splenectomy.

Date.	Weight.	N intake.*	Urine.								Feces total N.	N of urine and feces.	N balance.
			Amount.	Specific gravity.	Reaction to litmus.	H ion concentration.**	Total N.	Ammonia N.	Creatinine.	Creatine.			
	kilos	*gm.*	*cc.*	10			*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Nov. 16	13.4	5.60	175	56	Acid	6.70	5.35	0.38	0.300	0.358	0.34	5.69	−0.09
" 17	13.4	5.60	250	47	"	6.90	5.48	0.37	0.338	0.195	0.34	5.82	−0.22
" 18	13.4	5.60	225	50	"	6.70	4.91	0.38	0.352	0.480	0.34	5.25	+0.35
" 19	13.4	5.60	210	43	"	6.80	5.33	0.37	0.368	0.427	0.34	5.67	−0.07
" 20	13.4	5.60	200	44	"	6.90	4.80	0.34	0.361	0.467	0.34	5.14	+0.46
" 21	13.4	5.60	235	46	"	6.90	4.91	0.31	0.368	0.408	0.34	5.25	+0.35
" 22	13.3	5.60	265	37	"	6.90	4.77	0.27	0.385	0.597	0.34	5.11	+0.49
Average..	13.4	5.60	223	46		6.83	5.08	0.35	0.353	0.419	0.34	5.42	+0.18
Nov. 24	Splenectomy.												
Dec. 7	13.2	5.60	195	55	Acid	7.14	5.19	0.31	0.340	0.575	0.30	5.49	+0.11
" 8	13.1	5.60	280	35	"	6.00	5.36	0.34	0.368	0.513	0.30	5.66	−0.06
" 9	13.1	5.60	290	33	"	6.90	4.95	0.29	0.352	0.636	0.30	5.25	+0.35
" 10	13.1	5.60	265	39	"	6.80	4.65	0.29	0.368	0.513	0.30	4.96	+0.64
" 11	13.1	5.60	235	38	"	6.80	4.38	0.29	0.324	0.527	0.30	4.68	+0.92
" 12	13.1	5.60	295	36	"	6.80	4.40	0.27	0.329	0.558	0.30	4.70	+0.90
" 13	13.1	5.60	245	35	"	6.80	4.52	0.23	0.385	0.672	0.30	4.82	+0.78
Average..	13.1	5.60	256	39		6.75	4.78	0.29	0.352	0.570	0.30	5.08	+0.51
Jan. 18	13.6	5.70	275	30	Acid	6.80	4.46	0.22	0.355	0.398	0.40	4.86	+0.84
" 19	13.6	5.70	210	44	"	6.80	4.32	0.24	0.346	0.382	0.40	4.72	+0.98
" 20	13.6	5.70	310	20	"	6.70	4.58	0.22	0.364	0.361	0.40	4.98	+0.72
" 21	13.5	5.70	300	39	"	6.80	4.92	0.21	0.355	0.474	0.40	5.32	+0.38
" 22	13.5	5.70	250	35	"	6.70	4.49	0.24	0.311	0.362	0.40	4.89	+0.81
" 23	13.4	5.70	300	39	"	6.90	5.12	0.23	0.337	0.463	0.40	5.52	+0.18
" 24	13.4	5.70	300	39	"	6.90	5.51	0.22	0.326	0.405	0.40	5.91	−0.21
Average..	13.5	5.70	278	35		6.80	4.77	0.22	0.342	0.406	0.40	5.47	+0.53

* Diet: raw beef heart, 200 gm.; lard, 60 gm.; sugar, 60 gm.

** Expressed as negative logarithms.

the lard was cut out of the diet the animal ate readily, and later when the lard was again added no trouble was experienced. As may be seen in Table V, this was the only animal which showed a high neutral fat content in the feces, though what relation there may be between this and the dislike of fat is not evident. The practical result of this loss of weight after splenectomy was a moderate retention of nitrogen in the first postsplenectomy metabolism period.

TABLE III.

Dog 52. Nitrogen Metabolism before and Two, Six, and Ten Weeks after Splenectomy.

Date.	Weight.	N intake.*	Urine.								Feces total N.	N of urine and feces.	N balance.
			Amount.	Specific gravity.	Reaction to litmus.	H ion concentration.**	Total N.	Ammonia N.	Creatinine.	Creatine.			
	kilos	*gm.*	*cc.*	10			*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Nov. 25	10.8	4.77	170	44	Acid	6.50	4.17	0.30	0.368	None	0.38	4.58	+0.22
" 26	10.8	4.77	160	44	"	6.50	4.23	0.26	0.378	"	0.38	4.61	+0.16
" 27	10.8	4.77	225	33	"	6.50	4.49	0.23	0.368	"	0.38	4.87	−0.10
" 28	10.8	4.77	170	58	"	6.50	4.55	0.24	0.368	"	0.38	4.93	−0.16
" 29	10.8	4.77	180	46	"	6.50	4.55	0.25	0.368	"	0.38	4.93	−0.16
" 30	10.8	4.77	215	40	"	6.50	4.68	0.25	0.358	"	0.38	5.06	−0.29
Dec. 1	10.8	4.77	160	43	"	6.50	4.44	0.25	0.351	"	0.38	4.82	−0.05
Average..	10.8	4.77	183	44		6.50	4.44	0.25	0.365		0.38	4.82	−0.05
Dec. 2	Splenectomy.												
" 15	9.4	4.70	225	23	Acid	6.90	2.75	0.16	0.213	None	0.44	3.19	+1.51
" 16	9.4	4.70	180	39	"	6.50	3.73	0.25	0.324	"	0.44	4.17	+0.53
" 17	9.5	4.70	115	43	"	6.80	2.99	0.15	0.281	"	0.44	3.43	+1.27
" 18	9.5	4.70	210	35	"	6.80	2.79	0.15	0.289	"	0.44	3.23	+1.47
" 19	9.6	4.70	85	53	"	6.50	2.48	0.20	0.295	"	0.44	2.92	+1.78
" 20	9.7	4.70	275	20	"	6.70	2.79	0.25	0.291	"	0.44	3.23	+1.47
" 21	9.8	4.70	175	30	"	6.70	2.52	0.23	0.289	"	0.44	2.96	+1.74
" 22	9.8	4.70	225	25	"	6.80	2.88	0.25	0.311	"	0.44	3.32	+1.38
Average..	9.6	4.70	186	34		6.71	2.87	0.21	0.287		0.44	3.31	+1.39
Jan. 12	10.3	4.40	235	28	Acid	6.00	3.33	0.27	0.311	None	0.48	3.81	+0.59
" 13	10.3	4.40	190	35	"	6.30	3.38	0.24	0.311	"	0.48	3.86	+0.54
" 14	10.3	4.40	210	35	"	6.15	3.60	0.26	0.311	"	0.48	4.08	+0.32
" 15	10.4	4.40	180	42	"	6.30	3.45	0.22	0.324	"	0.48	3.93	+0.47
" 16	10.4	4.40	175	39	"	6.30	3.54	0.25	0.324	"	0.48	4.02	+0.38
" 17	10.4	4.40	225	35	"	6.70	3.38	0.25	0.311	"	0.48	3.86	+0.54
" 18	10.4	4.40	205	29	"	6.30	3.57	0.26	0.311	"	0.48	4.05	+0.35
Average..	10.35	4.40	203	35		5.29	3.46	0.25	0.318		0.48	3.94	+0.45
Feb. 10	10.8	4.10	160	35	Acid	6.90	2.96	0.23	0.225	None	0.44	3.40	+0.70
" 11	10.8	4.10	215	25	"	6.80	3.11	0.25	0.228	"	0.44	3.55	+0.55
" 12	10.8	4.10	220	22	"	6.80	3.23	0.20	0.234	"	0.44	3.67	+0.43
" 13	10.8	4.10	200	27	"	6.80	3.20	0.19	0.234	"	0.44	3.64	+0.46
" 14	10.9	4.10	190	32	"	6.80	3.44	0.18	0.231	"	0.44	3.88	+0.22
Average..	10.8	4.10	197	28		6.82	3.19	0.21	0.230		0.44	3.63	+0.47

* Diet: boiled beef heart, 100 gm.; lard, 50 gm.; sugar, 50 gm.

** Expressed as negative logarithms.

However, in the third period, when the animal had returned to exactly the same weight as before operation, nitrogen equilibrium

TABLE IV.

Dog 56. Nitrogen Metabolism before and Ten Days and Three Months after Splenectomy.

Date.	Weight.	N intake.*	Urine.								Feces total N.	N of urine and feces.	N balance.
			Amount.	Specific gravity.	Reaction to litmus.	H ion concentration.**	Total N.	Ammonia N.	Creatinine.	Creatine.			
	kilos	*gm.*	*cc.*	10			*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Feb. 16	8.5	3.43	100	39	Acid	6.30	2.63	0.13	0.221	0.014	0.35	2.98	+0.45
" 17	8.5	3.43	115	44	"	6.00	2.61	0.17	0.270	0.022	0.35	2.96	+0.47
" 18	8.5	3.43	140	38	"	6.15	2.39	0.14	0.279	0.022	0.35	2.74	+0.66
" 19	8.4	3.43	120	42	"	6.30	2.63	0.17	0.319	0.057	0.35	2.98	+0.45
" 20	8.4	3.43	120	47	"	6.30	2.73	0.16	0.261	0.116	0.35	3.08	+0.35
" 21	8.4	3.43	200	39	"	6.30	2.61	0.13	0.265	0.084	0.35	2.96	+0.47
" 22	8.4	3.43	90	50	"	6.15	2.60	0.14	0.253	0.067	0.35	2.95	+0.48
Average..	8.4	3.43	126	43		6.21	2.60	0.15	0.267	0.055	0.35	2.95	+0.48
Feb. 23	Splenectomy.												
Mar. 5	8.0	3.37	165	47	Acid	6.15	3.11	0.13	0.231	0.121	0.41	3.52	−0.15
" 6	8.0	3.37	110	46	"	6.15	3.00	0.14	0.213	0.143	0.41	3.41	−0.04
" 7	8.0	3.37	150	44	"	6.50	3.19	0.14	0.234	0.081	0.41	3.60	−0.04
" 8	8.0	3.37	210	40	"	6.15	3.30	0.16	0.221	0.119	0.41	3.71	−0.34
" 9	7.9	3.37	170	42	"	6.15	3.32	0.16	0.213	0.144	0.41	3.73	−0.36
" 10	7.9	3.37	130	49	"	5.85	3.18	0.16	0.213	0.114	0.41	3.59	−0.22
" 11	7.9	3.37	170	38	"	6.15	3.09	0.12	0.213	0.129	0.41	3.50	−0.13
Average..	8.0	3.37	152	44		6.14	3.17	0.14	0.220	0.122	0.41	3.72	−0.18
May 20	7.9	3.78	130		Acid		2.17		0.213	0.029	0.38	2.55	+1.23
" 21	7.9	3.78	145		"		2.07		0.213	0.014	0.38	2.45	+1.33
" 22	7.9	3.78	130		"		2.24		0.225	0.010	0.38	2.62	+1.16
" 23	7.9	3.78	260		"		2.56		0.238	0.017	0.38	2.94	+0.84
" 24	7.9	3.78	115		"		2.47		0.213	0.014	0.38	2.85	+0.93
Average..	7.9	3.78	156				2.30		0.220	0.017	0.38	2.68	+1.10

* Diet: boiled beef heart, 75 gm.; lard, 40 gm.; sugar, 40 gm.

** Expressed as negative logarithms.

was again maintained. It would seem conclusive, therefore, that the loss of weight and nitrogen retention of the earlier periods were due to an influence other than the absence of the spleen. It is of interest that this dog excreted no creatine.

In the experiments thus far presented there is no evidence that the absence of the spleen influences in any way nitrogen metabolism. In a fourth animal, however, the results were discordant.

This animal (Table IV) had served as a control for the blood

TABLE V.

Fat Determinations before and after Splenectomy.

Dog No.	Period.*	Total intake.	Total output.	Fat utilized.	Total output of fatty acids including soaps.	Fatty acids in total output.	Total output of neutral fats.	Neutral fat in total output.
		gm.	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*
48	I (7 days)	460.6	26.54	94.2	22.45	84.4	4.09	15.6
	Nov. 24	Splenectomy						
	II (7 days)	460.6	20.92	95.5	18.11	86.5	2.81	13.5
	III (7 days)	460.6	15.77	96.6	11.51	73.0	4.26	27.0
52	I (7 days)	374.8	9.19	97.5	6.29	68.4	2.90	31.6
	Dec. 2	Splenectomy						
	II (8 days)	428.32	12.64	97.0	5.93	46.9	6.71	53.1
	III (7 days)	374.8	17.14	95.4	8.44	49.2	8.70	50.8
	IV (5 days)	267.7	7.85	97.1	6.71	85.5	1.14	14.5
56	I (7 days)	298.4	13.59	95.4	9.27	68.3	4.32	31.7
	Feb. 23	Splenectomy						
	II (7 days)	298.4	14.25	95.2	11.11	78.0	3.14	22.0
57	I (7 days)	380.45	23.87	93.7	19.42	81.4	5.04	18.6
	Mar. 7	Splenectomy						
	II (7 days)	380.45	10.56	97.2	7.68	72.7	2.88	27.3

* These periods correspond exactly to those in Tables I, II, III, and IV.

counts of the three animals discussed above, and up to the time of our foreperiod had been for twelve weeks on an adequate constant diet, as was the case in the other animals. Like Dog 52 this animal received boiled meat as a part of the dietary. The effect of splenectomy on the nitrogen metabolism, ten days after the operation, was very slight, but a nitrogen equilibrium of + 0.47 of a gram per day was changed to one of — 0.18 of a gram, figures not beyond the range of normal variations, but which in the light of changes to be discussed later in the paper are suggestive of the influence of anemia. At a later period, three months after splenectomy, the animal had not regained the slight loss (0.5 of a kilo) in weight, but it appeared to

be in excellent condition and the anemia which had existed for several months was improving. The plus balance of 1.1 grams of nitrogen per day (upon a slightly higher nitrogen intake) in this period without change in weight suggests the possibility of the utilization of this nitrogen for the repair of the anemia. Utilization of protein was not disturbed, being 90 per cent in Period I, 88 per cent in Period II, and 90 per cent in Period III.

Unlike the other three dogs, we had here in Period II an increase of creatine amounting to 45 per cent. This increase was at the expense of the creatinine, however, for the total creatinine, including preformed creatinine and creatine as creatinine, agrees very closely, amounting to 0.314 of a gram in the foreperiod and 0.325 of a gram in the afterperiod.

During Period III, while the average creatinine output was exactly the same as during Period II, the creatine output fell to a figure lower than either of the preceding periods. The variation in the partition of creatine and creatinine in this animal we are unable to explain.

Fat Utilization.—The utilization of fat (Table V) in all the animals was normal in all periods. The partition of fatty acids (including soaps) and neutral fats shows some variation, especially in Dog 52, but to this we are inclined to ascribe no importance. A thorough search of the literature shows that no studies of fat utilization in animals before and after splenectomy have previously been made.

Iron Elimination.—The elimination of iron in the feces (Table VI) showed no important change after splenectomy in three of the four animals studied. In the fourth (Dog 56) there was an increase of 1.6 mg. per day during the period immediately after splenectomy, amounting to an increase of 21.6 per cent over the foreperiod. In the final period three months after splenectomy the output showed an increase of 148 per cent over the foreperiod and double that of Period II. The intake of iron was not determined, but since the food intake was constant throughout all the periods we have reason to believe that this was a constant factor. The periods of Table VI correspond exactly to those in previous tables. The

figures for iron are based on the analyses of samples of feces representing the total feces of the respective periods.

TABLE VI.

Iron Elimination before and after Splenectomy.

Dog No.	Before splenectomy.	After splenectomy.		
	Period I.	Period II.	Period III.	Period IV.
48	17.6*	17.9	16.8	
52	10.5	10.4	9.8	9.9
56	7.4	9.0	18.4	
57	10.9	10.8		

* The figures in this table represent mg. of iron per day in feces.

These studies of iron metabolism are in accord with those of Austin and Pearce (13) reported from this laboratory a year ago, but are opposed to those of Asher and his associates (14). The latter claim that after splenectomy the output of iron in the dog is much higher, often double that of the normal dog. Austin and Pearce, using the Ripper-Schwarzer method, found a slightly increased elimination in three of five dogs within two weeks after splenectomy, but no change in two others of this period or in three dogs studied 1, 9, and 20 months after splenectomy. The results now presented, by Neumann's method, appear to support the conclusion of Austin and Pearce that the spleen does not exert a constant and important influence upon iron metabolism.

DISCUSSION.

These observations show that in three of four animals the removal of the spleen had no effect upon nitrogen metabolism, the utilization of fat, or the elimination of iron, and justify the conclusion that the removal of the normal spleen in a normal animal has no important effect upon general metabolism. It is necessary, however, in order that there may be no question about this conclusion to explain the discordant results in the fourth animal (Dog 56). This animal showed a loss of weight, an increased elimination of iron, and a disturbance of creatine metabolism. The fat metabolism was unaltered. The question arises whether these changes are due to the absence of the spleen, or to the anemia which was present.

In Table VII are presented the blood examinations of each dog at the time of the several metabolism periods. It will be seen that Dogs 48 and 57 showed no appreciable change in the blood picture after splenectomy, but that Dogs 52 and 56 did. The blood changes in Dog 52, however, were relatively slight. The situation in regard

TABLE VII.

*Blood Examinations.**

Dog No.	Period.**	Hemoglobin.	Red cell count.
		per cent	
48	I	100	5,900,000
	II	104	5,570,000
	III	100	5,540,000
52	I	105	6,700,000
	II	106	6,840,000
	III	84	5,360,000
	IV	90	5,100,000
56	Initial	105	6,450,000
	I	83	6,020,000
	II	70	5,890,000
	III	72	4,950,000
57	I	95	6,130,000
	II	90	6,130,000

* These examinations were made by Dr. J. H. Austin.

** The periods correspond exactly to those of the previous tables.

to Dog 56 was somewhat different. This animal had been placed on a constant diet, the chief article of which was boiled beef heart, twelve weeks before the first metabolism study. At that time the blood examination showed hemoglobin 105 per cent and red cells 6,450,000. At the time of our presplenectomy period it showed a relatively low hemoglobin content (83 per cent) and 6,020,000 red cells; and after splenectomy the hemoglobin continued to fall, and later examinations, not corresponding to a metabolism period, showed the low level, two and a half months after splenectomy, of hemoglobin 60 per cent, and red cells 4,560,000. It is evident, therefore, that this animal differed from the other three in that it developed an anemia more rapidly and eventually of a more severe grade than was the case in any other animal of this series. As has been shown in previous publications (15) from this laboratory,

anemia of varying severity is a fairly constant result of splenectomy in the dog. The anemia may be slight as in Dog 52, or more severe as in Dog 56, and may, as has been suggested, be prevented by diet. The influence of diet is one of the problems now under investigation in this laboratory, but is not a matter which concerns us at the present time. The essential fact is that Dog 56 developed a severe anemia, already progressive at the time of the first metabolism study, while Dogs 48 and 57 were not anemic, and Dog 52 showed only a slight non-progressive deterioration of the blood. The question naturally arises: Are the increased elimination of iron and the disturbance of the creatine metabolism due to the anemia and not to an influence on metabolism consequent upon the absence of the spleen?

A few words are necessary concerning Richet's statement that the splenectomized dog requires more food to maintain its weight than does the normal dog. In view of our results, Richet's conclusion is not tenable. Dog 57 (Table I) maintained its presplenectomy weight without change in diet and with only a slight change in the nitrogen balance. Dog 48 (Table II) likewise showed only a trifling change during the three weeks after operation and a return to the previous weight after seven to eight weeks. The serious loss of weight in Dog 52 was due to loss of appetite, and that, relatively slight, in Dog 56 was complicated by the coexisting anemia.

Moreover, during the past five years we have frequently noticed a tendency for splenectomized dogs to become obese, and this tendency is mentioned also by several investigators who had studied splenectomized animals for long periods of time. This tendency to put on weight is strikingly shown by two of the dogs (48 and 52) of this series. At the close of the metabolism work presented in Tables II and III these animals were kept for some time on account of the possible necessity of repeating the metabolism studies after longer periods had elapsed. The change from a special diet to the ordinary kennel diet ("table scraps") led to a rapid increase of weight in each instance; in three months the weight of Dog 48 increased from 13.4 to 15.8 kilos, while in two months Dog 52 rose from 10.9 to 12.9 kilos.

Our results are therefore in accord with those of Paton rather than with those of Richet, and demonstrate that in the absence of anemia the removal of the spleen has no influence upon nitrogen or fat metabolism, and in all probability no influence upon iron elimination.

SUMMARY.

Four dogs have been subjected to metabolism studies before splenectomy and at intervals of three days to three months after splenectomy. In three of the four animals the removal of the spleen was not followed by any disturbances of nitrogen metabolism, fat utilization, or iron elimination. Two of these animals showed no anemia, and the third only a slight reduction in hemoglobin and number of red cells.

A fourth animal, studied ten days and three months after splenectomy, developed eventually a definitely progressive anemia of moderate severity. This animal showed a slight loss of weight, a slight disturbance of nitrogen balance, and of creatine-creatinine partition, with a marked increase in the elimination of iron.

We conclude therefore that the spleen has no important influence on metabolism, and that the disturbances occurring in one of our dogs were due to the coexisting anemia and not to the absence of the spleen.

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13. Austin, J. H., and Pearce, R. M., The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. XI. The Influence of the Spleen on Iron Metabolism, *Jour. Exper. Med.*, 1914, xx, 122.
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STUDIES IN LIVER AND KIDNEY FUNCTION IN EXPERIMENTAL PHOSPHORUS AND CHLOROFORM POISONING.

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(Received for publication, May 25, 1915.)

INTRODUCTION.

In the course of a somewhat extended study of the value of various tests of liver function in clinical diseases of the liver,[1] it seemed advisable to determine whether a rough approximate standardization of the functional changes could be obtained through comparison of the findings in clinical conditions with graded experimental liver injuries in animals.

Chloroform and phosphorus poisoning have probably been most used in producing experimental liver lesions. The non-specificity of the injury to the liver is at once apparent in the consideration of this method, for it is well known that pathological changes in the kidneys, heart, suprarenals, and voluntary muscles occur as the results of these poisons. The general opinion that the liver lesions are the predominating pathological features and that they are responsible for certain metabolic changes led us to investigate these conditions. However, it may be stated at once that we feel that deductions concerning liver function drawn from such studies are not free from criticism, and may be applied to findings in clinical material only with reserve.

An acceleration of protein metabolism, as evidenced by increased nitrogen output in the urine, has been shown to occur in phosphorus poisoning by Bauer,[2]

[1] Rowntree, L. G., Marshall, E. K., Jr., and Chesney, A. M., *Tr. Assn. Am. Phys.*, 1914, xxix, 586. Chesney, Marshall, and Rowntree, *Jour. Am. Med. Assn.*, 1914, lxiii, 1533.

[2] Bauer, J., *Ztschr. f. Biol.*, 1871, vii, 63; 1878, xiv, 527.

and in chloroform poisoning by Strassmann.[3] This has been generally confirmed by all subsequent workers.[4] It has been attributed to a decrease in the oxidation processes in the body and to autolysis of tissues.[5] However, Lusk[6] has shown that there is no reduction in the amount of total metabolism in phosphorus poisoning, but there may be rather an increase due to fever and the increased protein metabolism.

In general, the changes occurring in the urine and blood in phosphorus and in chloroform poisoning are similar to those which seem to be characteristic of certain types of liver disease in man.[7] Vidal[8] showed a slight decrease in the urea, and increase in the uric acid, creatinine, and undetermined nitrogen percentages of the total in experimental chloroform poisoning. Paton[9] distinguished between the effects of its inhalation and of its administration by the stomach. In the former he found a normal or increased urea and normal or decreased ammonia percentage; in the latter, a decided decrease in the urea and increase in the ammonia, uric acid, and undetermined nitrogen percentages of the total. He did not think the discrepancy due to size of the dose. Given hypodermically, the same effect, though less pronounced, was noted as when administered by mouth. Howland and Richards,[10] studying delayed chloroform poisoning (anesthesia) in dogs, noted slightly decreased urea and increased ammonia and undetermined nitrogen percentages of the total. Lindsay[11] confirmed these results and found the allantoin, creatine, amino-acids, and polypeptides increased.

The excretion of amino-acids in the urine in phosphorus poisoning has been shown by Abderhalden and Bergell[12] and Wohlgemuth.[13] Fischler and Bardach[14] found in dogs, with and without an Eck fistula, no difference in the nitrogen partition in phosphorus poisoning, low urea and high ammonia percentages, and increase in formol-titratable nitrogen in both. Ishihara[15] has recently studied

[3] Strassmann, F., *Virchows Arch. f. path. Anat.*, 1889, cxv, 1.

[4] Taniguti, K., *Virchows Arch. f. path. Anat.*, 1890, cxx, 121. Savelieff, N., *Virchows Arch. f. path. Anat.*, 1894, cxxxvi, 195. Vidal, E., Influence de l'anesthésie chloroformique sur les phenomènes chimiques de l'organisme, Thèse de Paris, 1897. Paton, D. N., *Proc. Roy. Soc. Edinburgh*, 1908, xxviii, 472. Howland, J., and Richards, A. N., *Jour. Exper. Med.*, 1909, xi, 344. Lindsay, D. E., *Biochem. Jour.*, 1910–11, v, 407.

[5] Jacoby, M., *Ztschr. f. physiol. Chem.*, 1900, xxx, 149. Waldvogel, *Arch. f. klin. Med.*, 1905, lxxxii, 437. Wells, H. G., *Jour. Biol. Chem.*, 1908–09, v, 129.

[6] Lusk, G., *Am. Jour. Physiol.*, 1907, xix, 461. Mandel, A. R., and Lusk, G., *Am. Jour. Physiol.*, 1906, xvi, 129.

[7] For a full discussion of the functional changes occurring in liver diseases, see Rowntree, Marshall, and Chesney, *loc. cit.* Here also will be found a full discussion of the methods employed in our liver functional studies.

[8] Vidal, *loc. cit.*

[9] Paton, *loc. cit.*

[10] Howland and Richards, *loc. cit.*

[11] Lindsay, *loc. cit.*

[12] Abderhalden, E., and Bergell, P., *Ztschr. f. physiol. Chem.*, 1903, xxxix, 464.

[13] Wohlgemuth, J., *Ztschr. f. physiol. Chem.*, 1905, xliv, 74.

[14] Fischler, F., and Bardach, K., *Ztschr. f. physiol. Chem.*, 1912, lxxviii, 435.

[15] Ishihara, H., *Biochem. Ztschr.*, 1912, xli, 315.

subchronic phosphorus poisoning in dogs, and could demonstrate no noticeable changes in the distribution of the nitrogen between the creatine, creatinine, amino-acids, and ammonia.

The most noticeable changes which have been shown in the blood are the marked decrease in fibrinogen and increase in the lipolytic activity. Corin and Ansiaux[16] and Jacoby[17] found the blood incoagulable in phosphorus poisoning, while this decrease in fibrinogen was shown by Doyon and Billet[18] for chloroform poisoning. Whipple[19] and his coworkers have shown the increase in the lipolytic activity of the blood in these conditions, and have further made an extensive study on lipase and fibrinogen in experimental liver injuries (chloroform, phosphorus, hydrazine poisoning, etc.), and in certain clinical diseases of the liver. Ragazzi[20] found that the viscosity and electrical conductivity of the blood were increased in phosphorus poisoning. Frank and Isaac[21] found the blood sugar to be decreased to z. .o shortly before death from phosphorus poisoning. Opie, Barker, and Dochez[22] showed an increase in the proteolytic enzymes of the blood after chloroform and phosphorus poisonings.

Simultaneous clinical studies by Rowntree, Hurwitz, and Bloomfield,[23] and experimental studies by Whipple, Peightal, and Clark[24] were carried on to determine whether the excretion of phenoltetrachlorphthalein by the bowels in health and disease afforded information relative to liver function. Whipple's studies revealed the facts that in chloroform and phosphorus poisonings marked decrease in the phthalein output resulted, together with the appearance of the drug in the urine, and further that, associated with decrease in the phthalein output, decrease in fibrinogen and increase in blood lipase occurred. In the recent study of lactose tolerance in chloroform and phosphorus poisonings in dogs, Bloomfield and Hurwitz[25] have reviewed the literature on sugar tolerance.

Methods.

Before inducing the chloroform or phosphorus poisoning, each dog was subjected to the series of tests to establish its normals. No

[16] Corin, G., and Ansiaux, G., *Jahresb. f. Thierchem.*, 1894, xxiv, 642.

[17] Jacoby, *loc. cit.*

[18] Doyon, M., and Billet, J., *Compt. rend. Soc. de biol.*, 1905, lvii, 852.

[19] Whipple, G. H., Mason, V. R., and Peightal, T. C., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 207. Whipple, G. H., and Hurwitz, S. H., *Jour. Exper. Med.*, 1911, xiii, 136. Whipple, G. H., *Arch. Int. Med.*, 1912, ix, 365.

[20] Ragazzi, C., *Jahresb. f. Thierchem.*, 1910, xl, 1276.

[21] Frank, E., and Isaac, S., *Arch. f. exper. Path. u. Pharmakol.*, 1911, lxiv, 274.

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[24] Whipple, G. H., Peightal, T. C., and Clark, A. H., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 343.

[25] Bloomfield, A. L., and Hurwitz, S. H., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 375.

attempt was made to maintain nitrogenous equilibrium except in two animals, although in the others the diet was kept fairly constant throughout the period of observation.[26] The chloroform was given by inhalation, and the phosphorus subcutaneously, in oil. The phenoltetrachlorphthalein test was employed, according to the technique of Rowntree, Hurwitz, and Bloomfield.[27] The fibrinogen determinations were made by the heat coagulation method of Whipple,[28] and the lipolytic activity of the blood plasma by Loevenhart's[29] method. The total non-protein nitrogen, urea nitrogen, and amino-acid nitrogen of the blood serum or whole blood were estimated by the micro-Kjeldahl method of Folin and Denis,[30] the urease method of Marshall,[31] and the nitrous acid method of Van Slyke,[32] respectively. In the urine, total nitrogen was determined by the usual Kjeldahl, the urea by Marshall's,[33] the ammonia by Folin's,[34] and the free amino-acids by Van Slyke's[35] method. The tolerance towards levulose and galactose was determined in the usual way. The hydrogen ion concentration of the blood was determined by the method recently described by Levy, Marriott, and Rowntree.[36]

Liver Function in Phosphorus Poisoning.

In the following protocols of the various animals, the fibrinogen, total non-protein nitrogen, urea nitrogen, and amino nitrogen are expressed in mg. per 100 cc. of blood serum. The urine quantity, unless otherwise stated, is for 24 hours, and the urea nitrogen, ammonia nitrogen, and free amino nitrogen are expressed as percentages of the total nitrogen of the urine. The lipolytic activity of the blood

[26] In the more severe stages of poisoning, the animals refused food for several days.

[27] Rowntree, Hurwitz, and Bloomfield, *loc. cit.*

[28] Whipple, G. H., *Am. Jour. Physiol.*, 1914, xxxiii, 50.

[29] Loevenhart, A. S., *Am. Jour. Physiol.*, 1901–02, vi, 331.

[30] Folin, O., and Denis, W., *Jour. Biol. Chem.*, 1911–12, xi, 527.

[31] Marshall, E. K., Jr., *Jour. Biol. Chem.*, 1913, xv, 487.

[32] Van Slyke, D. D., and Meyer, G. M., *Jour. Biol. Chem.*, 1912, xii, 399.

[33] Marshall, E. K., Jr., *Jour. Biol. Chem.*, 1913, xiv, 283; xv, 495.

[34] Folin, O., *Ztschr. f. physiol. Chem.*, 1902–03, xxxvii, 161.

[35] Van Slyke, D. D., *Jour. Biol. Chem.*, 1913, xvi, 125.

[36] Levy, R. L., Marriott, W. M., and Rowntree, L. G., *Arch. Int. Med.*, 1915, xvi (in press).

plasma is expressed in the number of cc. of N/10 acid produced in 24 hours at 38° C. by a mixture of 1 cc. of plasma, 4 cc. of water, and 0.26 cc. of ethyl butyrate.

Dog I.—Weight 12 kilos. *Normals:* tolerates 0.3 gm. galactose and 1.5 gm. levulose per kilo. Phthalein 32 per cent. *Blood:* fibrinogen, 490 mg.; total non-protein N, 44 mg.; urea N, 20 mg. (46 per cent); amino N, 3.7 mg. *Urine:* 175 cc. containing 4.4 gm. nitrogen; urea N, 83 per cent; ammonia N, 7.4 per cent; amino N, 0.45 per cent.

Mar. 6, 1914. Given 10 mg. phosphorus in oil subcutaneously.

Mar. 8. Given 7.5 mg. phosphorus. 0.2 gm. per kilo of galactose not tolerated. *Urine:* 450 cc. containing 10.3 gm. nitrogen; urea N, 84 per cent; ammonia N, 5.4 per cent; amino N, 0.3 per cent.

Mar. 9. Bled and phthalein injected at noon; died at 3 p. m. *Blood:* lipase, 1.55; fibrinogen, 13 mg.; total non-protein N, 91 mg.; urea N, 33 mg. (36 per cent); amino N, 13.4 mg.

Autopsy.—Liver: light brownish yellow in appearance, very friable, and soft. On section the parenchyma has a mottled appearance, areas of depression alternating with those which project. *Lungs:* pale and edematous.

Microscopic Examination.[37]*—Liver:* diffuse peripheral hemorrhagic necrosis. Necrotic cells are found scattered through all parts of the liver lobule. There are no normal liver cells seen. Those remaining alive show extreme parenchymatous and fatty degeneration.

Dog II.—Weight 23 kilos. *Normals:* tolerates 0.25 gm. but not 0.30 gm. galactose and 1.5 gm. levulose per kilo. Phthalein 31 per cent. *Blood:* lipase, 0.25; fibrinogen, 243 mg.; total non-protein N, 51 mg.; urea N, 20 mg. (39 per cent); amino N, 3.8 mg. *Urine:* 390 cc. containing 12.0 gm. nitrogen; urea N, 85 per cent; ammonia N, 5.3 per cent; amino N, 1.5 per cent.

Mar. 21, 1914. Given 13 mg. phosphorus.

Mar. 23. Given 5 mg. phosphorus.

Mar. 24. Phthalein 23 per cent positive in urine.

Mar. 26. Given 5 mg. phosphorus. Tolerates 0.20 gm. galactose per kilo. *Urine:* 600 cc. containing 12.1 gm. nitrogen; urea N, 84 per cent; ammonia N, 5.6 per cent; amino N, 0.99 per cent. Bile in urine, but no jaundice; dog seems sick.

Mar. 27. Given 3 mg. phosphorus.

Mar. 28. Tolerates 1.2 gm. levulose per kilo; phthalein 0 per cent; positive in urine. *Blood:* lipase, 0.60; fibrinogen, 10 mg.; total non-protein N, 110 mg.; urea N, 61 mg. (55 per cent); amino N, 11.3 mg. *Urine:* 1,200 cc. containing 18.2 gm. nitrogen; urea N, 77 per cent; ammonia N, 6.0 per cent; amino N, 4.6 per cent.

Mar. 29. Dog very sick; comatose. Died. *Blood:* (just after death) lipase, 1.30; total non-protein N, 225 mg.; urea N, 179 mg. (79 per cent); amino N, 21 mg. *Bladder urine:* 100 cc. containing 1.6 gm. nitrogen; urea N, 82 per cent; ammonia N, 4.7 per cent; amino N, 2.1 per cent.

Dog III.—Weight 22 kilos. *Normals:* tolerates 0.30 gm. galactose and 1.5

[37] For the microscopic reports we are indebted to Dr. G. H. Whipple.

gm. levulose per kilo. Phthalein 30 per cent. *Blood:* lipase, 0.35; fibrinogen, 330 mg.; total non-protein N, 28 mg.; urea N, 8 mg. (29 per cent); amino N, 3.4 mg. *Urine:* 533 cc. containing 10.6 gm. nitrogen; urea N, 81 per cent; ammonia N, 6.7 per cent; amino N, 1.4 per cent.

Mar. 18, 1914. Given 10 mg. phosphorus.

Mar. 19. Tolerates 0.20 gm. galactose per kilo.

Mar. 20. Given 8 mg. phosphorus.

Mar. 21. Tolerates 1.25 gm. levulose per kilo.

Mar. 23. Phthalein 6 per cent; lipase, 0.85; fibrinogen, 200 mg.; total non-protein N, 50 mg.; urea N, 22 mg. (44 per cent); amino N, 3.4 mg.

Mar. 26. *Urine:* 410 cc. containing 7.4 gm. nitrogen; urea N, 83 per cent; ammonia N, 7.4 per cent; amino N, 1.1 per cent.

Mar. 27. Playful. Given 7.5 mg. phosphorus.

Mar. 28. Playful. Does not tolerate 1.2 gm. levulose per kilo. Phthalein 17 per cent positive in urine.

Apr. 2. Does not tolerate 0.20 gm. galactose per kilo.

Apr. 3. Does not tolerate 1.0 gm. levulose per kilo. Given 8 mg. phosphorus.

Apr. 4. Phenolsulphonephthalein 50 per cent for 1¼ hours.

Apr. 5. Does not tolerate 0.18 gm. galactose per kilo. Phthalein 26 per cent. Apparently has been in splendid condition for past week.

Apr. 6. Given in a. m. 11 mg. phosphorus and in p. m. 8 mg. more. *Blood:* lipase, 0.75; fibrinogen, 400 mg.; total non-protein N, 42 mg.; urea N, 18 mg. (43 per cent); amino N, 2.0 mg.

Apr. 7. Vomited. Looks depressed. Wants to lie down. Phenolsulphonephthalein 44 per cent in 1½ hours. *Urine:* 530 cc. containing 8.5 gm. nitrogen; urea N, 82 per cent; ammonia N, 3.2 per cent; amino N, 1.7 per cent. Tolerates 0.8 gm. levulose per kilo.

Apr. 8. Found dead. Weight 17.4 kilos. Has not eaten anything for 2 to 3 days. *Blood:* does not coagulate; total non-protein N, 84 mg.; urea N, 28 mg. (33 per cent); amino N, 11.3 mg.

Autopsy.—Kidney: diffuse epithelial necrosis, involving particularly the convoluted tubules.

The tests on the three dogs suffering from phosphorus poisoning (I, II, and III) showed the following definite agreement in all: The phthalein excretion in the feces was markedly diminished and the dye appeared in the urine; the lipolytic activity of the blood was greatly increased; the fibrinogen sank to a very low level; the total non-protein nitrogen, urea nitrogen, and amino nitrogen of the blood were all more or less increased. The urines showed no conclusive changes in all, while the galactose and levulose tolerance was rather definitely decreased. The urea nitrogen percentage of the total non-protein nitrogen of the blood showed an increase in one case and decrease in the other two. However, this is not at all striking, and no conclusion concerning it can be drawn. The high

total non-protein nitrogen and urea nitrogen of the blood in Dog II are worthy of note. The highest blood amino nitrogen (21 mg. to 100 cc.) which we have encountered was seen in this case, in which also the partition of the urinary nitrogen was most markedly disturbed. However, with the possible exception of Dog III (March 23), this was the only time when a severe stage of poisoning (liver injury) as indicated by other tests and the general condition of the animals was coincident with a urine analysis. In Dog II we see the gradual drop of the phthalein to zero, and the liver injury, as shown by the test, was probably in this case the most severe. Dog III apparently had a remarkable resistance to phosphorus. The liver repair indicated on April 5 to 6 is worthy of note.

TABLE I.

Dog IV.

Date (1924).	Phosphorus.	Urine.					Total N.			Blood.			Weight.
		Amount.	Total N.	Urea N.	Ammonia N.	Amino N.	Urea N.	Ammonia N.	Amino N.	Total non-protein N.	Urea N.	Amino N.	
										Per 100 cc.			
	mg.	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*mg.*	*mg.*	*mg.*	*kilos*
Nov. 30		112	4.33	3.53	0.18		82	4.2					8.40
Dec. 2													
" 3	20	205	7.53	6.33	0.41	0.044	84	5.5	0.6	28	12	3.7	8.10
" 4	10	154	3.23	2.52	0.11	0.031	78	3.4	1.0				8.20
" 5		200	5.78	4.91	0.33	0.092	85	5.7	1.6				
" 6	20	218	8.74	6.21	0.26	0.055	72	3.0	0.7				
" 7										88	66	7.7	7.45
Dog V.													
Dec. 11		237	4.19	3.60	0.09		86	2.2					8.40
" 15		78	4.52	3.93		0.05	87		1.1				
" 16	20	110	6.60	5.61	0.76	0.03	85	4.0	0.5	29	12	4.3	8.45
" 17	5	170	4.66	3.90	0.16		83	3.6					
" 19										88	49	7.5	
" 20		115	2.00	1.42	0.16	0.14	71	8.0	7.0	140	90	13.2	7.25

Nitrogen Metabolism of Dogs in Phosphorus Poisoning.

It was considered advisable to obtain data on the nitrogen metabolism in phosphorus poisoning, in view of the cumulative phenomena observed in the blood. Information concerning the relation between the amount of the nitrogenous bodies in the blood and their excretion

in the urine is desirable particularly to determine if renal function is involved.

Two dogs (IV and V) were placed on a diet of 200 gm. of ground meat and 150 cc. of water until nitrogenous equilibrium was obtained. Two to three days prior to the administration of phosphorus, the urinary partition (urea N, ammonia N, and amino N) was determined, as well as the total non-protein N, urea N, and amino N of the whole blood. Phosphorus poisoning was induced, and the metabolic studies were continued, the results appearing in Table I.

Dog IV continued to eat until the day of death, whereas Dog V ate nothing for the last two days. Dog IV shows a marked increase in the output of urinary N, with a slight decrease in the urea nitrogen per cent, but no change in the ammonia and amino-acid N per cent, together with an absolute increase in total non-protein N, the urea N and amino N of the blood. Dog V exhibited practically normal urinary N figures until the day of death, when decrease in the total N, an absolute and relative decrease in urea N, and a relative increase in the ammonia and amino N percentages were found. Very marked accumulation of total non-protein N, urea, and amino N occurred in blood.

Renal Function in Phosphorus Poisoning in Dogs.

Since urinary nitrogen changes are utilized in studies of liver function, it is obviously necessary to have some information concerning the effect on renal function of drugs used for the production of these liver injuries. The cumulative phenomena in the blood encountered in our studies on liver function in phosphorus poisoning suggested that the kidney function was affected shortly before death. It was also considered advisable to determine whether an acidosis occurs, as this would affect conclusions drawn from the urinary ammonia findings. The phenolsulphonephthalein output in the urine, and the urea and hydrogen ion concentrations in the blood, were utilized in this connection. The results of studies on four dogs appear in Table II.

In these dogs sufficient phosphorus was given to insure definite functional and anatomical liver injury. Death from the phosphorus resulted in three of them, and jaundice and incoagulable blood were seen in all. These rarely appear except in fatal poisoning.

In Dogs VI and VII, acutely poisoned, no decrease in the phenolsulphonephthalein occurred. The blood urea increased only as a terminal event. Acidosis is indicated in each on the day before death.

Dog VIII, which recovered, showed at one time a phenolsulphonephthalein output considerably decreased,—28 per cent. However, the phthalein rapidly returned to normal. No marked increase in blood urea and no acidosis accompanied this change.

TABLE II.

Dog VI.

Date (1915).	Weight.	Phosphorus.	Sulphonephthalein, 2 hrs.	Blood urea per 100 cc.	Hydrogen ion concentration of blood.
	kilos	*mg.*	*per cent*	*mg.*	
Mar. 30			78		
" 31	8.0			24	7.70
Apr. 3			68		
" 7	8.4	12.5	70	32	7.45
" 8	8.55		55	35	7.65
" 9	8.40	5.0	80	40	
" 10			60	68	7.25
" 11				176	

Dog VII.

Date (1915).	Weight.	Phosphorus.	Sulphonephthalein, 2 hrs.	Blood urea per 100 cc.	Hydrogen ion concentration of blood.	Hydrogen ion concentration of serum.
	kilos	*mg.*	*per cent*	*mg.*		
Mar. 31	12.22		60	30	7.55	7.55
Apr. 3			60			
" 7	13.00	20		36	7.50	7.70
" 8	13.50		70	37	7.55	7.65
" 9	12.60	7.5	56	35		
" 10			75	35		
" 11			65	46	7.25	
				127 at death		

Dog VIII.

Date (1915).	Weight.	Phosphorus.	Sulphonephthalein, 2 hrs.	Blood urea per 100 cc.	Hydrogen ion concentration of blood.
	kilos	*mg.*	*per cent*	*mg.*	
Apr. 13			62	20	7.60
" 14	7.60	5			
" 16		5	67		7.45
" 17			75	40	
" 19		5		30	7.40
" 20				30	
" 21	6.35		58		
" 22			28	40	
" 23			45		7.45
" 26		5		36	7.60
" 27			63		
" 28		5			
" 29	6.30		75	51	7.60
" 30		5		36	
May 3	5.85		60	22	7.65
" 7			61	17	

TABLE II.—*Concluded.*

Dog IX.

Date (1915).	Weight.	Phosphorus.	Sulphone-phthalein, 2 hrs.	Blood urea per 100 cc.	Hydrogen ion concentration of blood.
	kilos	*mg.*	*per cent*	*mg.*	
Apr. 13			64	23	7.60
" 14	5.80	5			
" 16		5	75	30	7.45
" 17			80	27	
" 19				43	7.30
" 20				31	
" 21	5.35	5	73		
" 22			58		
" 23			50		7.35
" 26	5.25	5	65		
" 28		5			
" 29	5.15		53	55	7.55
" 30		5	45	32	7.60
May 3			20	79	7.35
" 4	4.75			112	

Dog IX exhibited a progressively decreasing renal function, as indicated by sulphonephthalein for the week previous to death, a normal increase in the blood urea, and an acidosis early in the poisoning, which reappeared before death.

That considerable injury to renal function does occur occasionally during the course of the intoxication, but that it is usually a terminal event, is evident from the study of these dogs. The renal injury, however, is exceedingly slight, and is late in comparison with the functional injury to the liver. The functional renal injury cannot be absolutely ignored, but it plays only a minor part in the interpretation of the functional changes of the liver resulting from the administration of phosphorus.

Liver Function in Chloroform Poisoning.

In the following protocols the results of liver studies similar to those carried out on the dogs poisoned with phosphorus are reported. No special renal functional studies, however, were made in chloroform poisoning.

Dog X.—Weight 24 kilos. *Normals:* tolerates 0.30 gm. galactose and 1.5 gm. levulose per kilo. Phthalein 30 per cent. *Blood:* lipase, 0.25; total non-protein N, 58 mg.; urea N, 30 mg. (52 per cent); amino N, 3.4 mg. *Urine:* 1,140 cc. containing 15.1 gm. nitrogen; urea N, 91 per cent; ammonia N, 5.8 per cent; amino N, 1.4 per cent.

Mar. 6, 1914. Chloroform anesthesia for 2 hours.

Mar. 8. Phthalein 22 per cent.

Mar. 10. Does not tolerate 0.20 gm. galactose per kilo.

Mar. 13. Tolerates 1.0 gm. levulose per kilo.

Mar. 14. *Blood:* lipase, 1.20; fibrinogen, 217 mg.; total non-protein N, 56 mg.; urea N, 26 mg. (46 per cent); amino N, 3.0 mg. *Urine:* 250 cc. containing 5.7 gm. nitrogen; urea N, 84 per cent; ammonia N, 6 per cent; amino N, 6.9 per cent.

Mar. 15. Died during chloroform anesthesia.

Dog XI.—Weight 16 kilos. *Normals:* phthalein 35 per cent. *Urine:* 425 cc. containing 7.9 gm. nitrogen; urea N, 82 per cent; ammonia N, 10 per cent; amino N, 1.1 per cent.

Mar. 3, 1914. Chloroform anesthesia for 2 hours.

Mar. 5. Found dead in cage. Body still very warm. *Bladder urine:* 100 cc., containing 2.2 gm. nitrogen; urea N, 40 per cent; ammonia N, 9.7 per cent; amino N, 3.0 per cent.

Dog XII.—Weight 8 kilos. *Normals:* tolerates 1.5 gm. levulose per kilo. Phthalein 34 per cent, positive in urine. *Blood:* lipase, 0.39; total non-protein N, 33 mg.; urea N, 11 mg. (33 per cent); amino N, 4.3 mg. *Urine:* 198 cc. containing 7.8 gm. nitrogen; urea N, 84 per cent; ammonia N, 6.4 per cent; amino N, 1.0 per cent.

Feb. 14. Chloroform anesthesia for 1½ hours.

Feb. 15. Phthalein 21 per cent, positive in urine. *Blood:* lipase, 0.93; fibrinogen, 395 mg.; total non-protein N, 52 mg.; urea N, 24 mg. (46 per cent); amino N, 4.9 mg.

Feb. 16. *Urine:* 265 cc., containing 13.4 gm. nitrogen; urea N, 84 per cent; ammonia N, 5.3 per cent; amino N, 1.5 per cent.

Died in subsequent chloroform anesthetization.

Dog XIII.—Weight 12 kilos. *Normals:* tolerates 0.22 gm. galactose and 1.5 gm. levulose per kilo. Phthalein 32 per cent. *Blood:* lipase, 0.38; fibrinogen, 485 mg.; total non-protein N, 39 mg.; urea N, 11 mg. (39 per cent); amino N, 4.8 mg. *Urine:* 360 cc. containing 9.1 gm. nitrogen; urea N, 83 per cent; ammonia N, 6.0 per cent; amino N, 1.2 per cent.

Mar. 19, 1914. Chloroform anesthesia for 2 hours. Very light. All subsequent ones light.

Mar. 23. Phthalein 25 per cent.

Mar. 25. Chloroform anesthesia for ¾ hour. Phthalein 27 per cent.

Mar. 26. Tolerates 0.16 gm. galactose per kilo.

Mar. 27. Chloroform anesthesia for 1 hour.

Mar. 28. Does not tolerate 1.2 gm. levulose per kilo.

Mar. 29. Phthalein 30 per cent.

Apr. 1. Chloroform anesthesia for 2 hours.

Apr. 2. Tolerates 0.16 gm. galactose per kilo.

Apr. 3. Does not tolerate 1 gm. levulose per kilo.

Apr. 4. Phthalein 9 per cent, positive in urine. Does not tolerate 0.16 gm. galactose per kilo.

Apr. 6. *Blood:* lipase, 0.85; fibrinogen, 14 mg.; total non-protein N, 25 mg.; urea N, 13 mg. (52 per cent); amino N, 4.0 mg.

Apr. 7. Tolerates 0.8 gm. levulose per kilo. Phthalein 2 per cent, positive in urine.

Apr. 8. *Blood:* lipase, 1.61; fibrinogen, 44 mg.; total non-protein N, 29 mg.; urea N, 10 mg. (34 per cent); amino N, 4.3 mg. *Urine:* 465 cc. containing 6.1 gm. nitrogen; urea N, 70 per cent; ammonia N, 14.4 per cent; amino N, 1.4 per cent.

Killed by bleeding from carotid.

Autopsy.—Liver: normal size, very pale, and friable. Lobules show very plainly. All tissues stained with bile. Kidney looks normal.

Microscopic Examination.—Kidney: cloudy swelling, moderate grade. Cells of convoluted tubules are swollen and granular. The lumina contain pink staining granules. *Liver:* shows central atrophy; extreme fatty degeneration, involving the entire lobule except a thin zone about the portal area; and numerous scattered necrotic liver cells, especially about the central portions of the lobules.

In order to gain some idea of the variations in normal dogs dependent on diet, Dogs XIV and XV were studied with uncontrolled diet (lungs, corn cake, etc.), and then on April 17 placed on a diet of 400 grams of meat per day. The total nitrogen and urea (whole blood) were taken at various times. Table III shows the variations encountered. The urea figures are expressed as mg. of nitrogen per 100 cc. of blood.

TABLE III.

Dog XIV.

Apr., 1914.	4	5	8	15	16	17	18	19	20	21	22	23	24	25	26	27
Total N, urine.....		4.4	9.0		11.2	10.0	5.8	20.0		13.6		15.2		10.1		
Urea N, blood.....	10		14	9	13			21	19				14			14

Dog XV.

Total N, urine.....		5.1	3.7		7.6	4.7	6.9	18.1		17.0		14.4		6.7		
Urea N, blood.....	11		14	9	15			20	25				18			18

Dog XIV.—Weight 11.9 kilos. *Normals:* phthalein 29 per cent. *Blood:* lipase, 0.35; fibrinogen, 470 mg.; total non-protein N, 34 mg.; urea N, 15 mg. (44 per cent); amino N, 3.7 mg.

May 1, 1914. Deep chloroform anesthesia for 2 hours.

May 2. Phenolsulphonephthalein between 60 and 70 per cent for 2 hours. *Blood:* lipase, 2.75; fibrinogen, 90 mg.; total non-protein N, 32 mg.; urea N, 13 mg. (40 per cent); amino N, 4.2 mg. *Urine:* single specimen; urea N, 44 per cent; ammonia N, 8.3 per cent; amino N, 3.1 per cent. Phthalein, only trace in feces.

Dog XV.—Weight 10.2 kilos. *Normals:* tolerates 0.3 gm. galactose and 1.5 gm. levulose per kilo. Phthalein 37 per cent.

May 5, 1914. Deep chloroform anesthesia for 1½ hours.

May 6. *Blood:* lipase, 2.70; fibrinogen, 30 mg.; total non-protein N, 120 mg.; urea N, 80 mg. (67 per cent); amino N, 6.6 mg. *Urine:* 24 hour specimen lost.

May 7. Dead. *Bladder urine:* urea N, 54 per cent; ammonia N, 10.6 per cent; amino N, 3.6 per cent. Phthalein 3 per cent.

In chloroform poisoning in dogs the following changes are found: The tetrachlorphthalein is decreased, and it appears in the urine; lipase is increased; fibrinogen is decreased; the total non-protein nitrogen, urea nitrogen, and amino nitrogen of the blood suffer practically no change. In the urine a more or less pronounced disturbance of the nitrogen partition is seen, while the galactose and levulose tolerance is definitely decreased.

Dog XV was the only one with chloroform poisoning which showed increase in nitrogenous products in the blood. Here, we have a condition somewhat resembling that in phosphorus dogs. The other tests show a very severe type of poisoning in this dog. This brings out the possibility of cumulative phenomena appearing in the course of chloroform poisoning. High amino-acid content of the urine is noted in Dog X (6.9 per cent); Dog XI (3.0 per cent); Dog XIV (3.1 per cent); and Dog XV (3.6 per cent). Dogs XI, XIV, and XV received poisonings which were rapidly fatal, dying within forty-eight hours. In them, a markedly disturbed nitrogen partition occurs in the urine, *i. e.,* a much decreased urea nitrogen, without a corresponding increase in the free amino-acids or ammonia nitrogen.

In Dog XIV, at a time when the liver function was reduced to a minimum, the sulphonephthalein was absolutely normal. It is very unlikely that renal functional changes are greater in chloroform than in phosphorus poisoning.

CONCLUSIONS.

1. We have confirmed the results of previous workers on tetrachlorphthalein, fibrinogen, and lipase.

2. The total non-protein nitrogen, urea, and amino-acids of the blood serum show a definite and sometimes marked increase in phosphorus poisoning. These changes are not so evident in chloroform poisoning, although they sometimes occur. They are usually terminal phenomena.

3. The urinary nitrogen partition between the urea, ammonia, and amino-acids is not always disturbed. The most important changes which occur are an increased amino nitrogen in chloroform and phosphorus poisoning, and a very low urea nitrogen percentage in severe fatal chloroform poisoning.

4. Sugar tolerance towards galactose and levulose is in general markedly decreased in both types of poisoning.

5. In phosphorus poisoning liver functional changes can and do occur without concomitant renal changes. Renal insufficiency usually arises as a terminal event.

6. Increased nitrogenous products in the blood (total non-protein nitrogen, urea, and amino nitrogen) are associated with an increase of these bodies in the urine. Consequently, an increased protein catabolism, as well as renal insufficiency, is necessary to explain this accumulation.

7. A terminal acidosis, as evidenced by increased hydrogen ion concentration in the blood, usually occurs.

THE INTOXICATION OF SPLENECTOMIZED MICE BY FEEDING FRESH SPLEEN AND OTHER ORGANS.

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(Received for publication, June 23, 1915.)

As we have previously recorded,[1] splenectomized mice when fed with fresh sheep or mouse spleen frequently show signs of illness. Some animals die within a few hours after eating the organ, and of those that die some show hemorrhages into the peritoneal subserosa or peritoneal cavity. The mice that have been ill are apt to refuse to eat the fresh organ on several succeeding days. When they do eat again they may or may not show signs of illness once more. Splenectomized mice fed with fresh sheep muscle never showed any sign of acute illness. Intact mice fed with spleen also showed no sign of ill effect.

These experiments were made on animals from two weeks to four weeks after the removal of the spleen. The intoxicating effect was readily noticeable in animals infected with the tubercle bacillus. In the non-infected animals the result was definite, but somewhat irregular, so much so that it seemed impracticable to do systematic work which should include a consideration of the negative results.

Passing by the steps which led to a definite and decisive method of work we may say that we found that at four or five days after splenectomy the intoxication is more regular in its occurrence and is apt to be more severe than at later periods. Also, as we continued these observations, we became impressed with the fact that the hemorrhage into the serous membranes and especially into the peritoneum, which we had occasionally noticed, was really an integral part of the process. Following a natural train of thought from this point we were led to test the coagulation time of the blood. We have found that the most constant result, following the feeding of

[1] Lewis, P. A., and Margot, A. G., *Jour. Exper. Med.*, 1915, xxi, 84.

spleen to splenectomized mice, is a delayed coagulation time. With these facts as a basis for judgment, we have studied the nature of this intoxication as it relates to the time of its possible development after splenectomy, the rate of its development after feeding, its specificity both as to organ and as to species, the amount of fresh substance necessary to produce the intoxication, and finally the effect of the continuous feeding of less than the fatal amount of substance.

Methods.

The general methods of splenectomy and feeding have been stated in our previous papers. With regard to the feeding we may repeat that the animals are fed with the fresh organs before other food is given on that day. We have found that the change in coagulation time is apt to be somewhat more pronounced after the second day's feeding than on the first day. Therefore, in case the first feeding has given a negative result, we have always fed with the same substance on the next day. The coagulation time has been taken with Bogg's instrument, the blood being drawn from the tail. In the following experiments the first feeding was on the fourth day after splenectomy. The second feeding was on the following day. The coagulation time was taken before feeding and usually from three to four hours after feeding.

The results of these experiments may be summarized as follows:

Splenectomized mice fed on the fourth day and again on the fifth day show no change in the coagulation time of the blood when the following organs are used: lymph nodes, thymus gland, liver, kidney, skeletal muscle, lung, brain, pancreas, pituitary gland, testicle, adrenal, thyroid gland, salivary glands, and the mucosa of the large intestines.

When fed with the following tissues the mice show a delay in the coagulation time varying between two minutes and fifteen minutes: spleen of mouse, rat, guinea pig, rabbit, beef, sheep, cat, and man; gastric mucosa of mouse and cat; and mucosa of the upper small intestines of mouse and cat.

When fed with fresh human bone marrow and with dried sheep blood the mice also show a change in coagulation time. In the experiments so far done the change has been less pronounced than after the feeding of spleen or gastro-intestinal mucosa.

The mice which show the smaller changes in coagulation time seldom show any other evidence of the intoxication. When the delay in coagulation time is marked the animals are apt to be extremely sick, and a considerable number of the animals have died. Death when it occurs usually takes place before the eighth hour. In twenty hours the blood of the surviving animals has usually returned to the normal. Occasionally animals are encountered in which the return to normal is less rapid. In these instances the blood coagulates slowly and the animal is still sick at the end of twenty-four hours. These animals sometimes recover and sometimes die. The animals which die during the course of this intoxication reveal various changes at postmortem examination. In some no gross changes can be made out. In others there are found petechial hemorrhages of the serous membranes, particularly the visceral peritoneum. Some instances of hemorrhage into the intestine have been encountered. The most usual result is a diffuse, rather poorly marked reddening of the peritoneal serosa accompanied by a small amount of blood tinged peritoneal fluid. Occasionally extremely large hemorrhages into the peritoneal cavity have occurred. When this has happened the animal has died quite suddenly. No definite bleeding point has been discovered in any instance. An intense diarrhea accompanies some cases.

We have been unable to distinguish in any way between the intoxication resulting from feeding spleen and that following the feeding of gastro-intestinal mucosa.

The tabulated results of the work on which the foregoing statements are based, together with the necessary control experiments, follow (Experiments I to XXX, Tables I to VIII).

TABLE I.

Experiment I. Rate of Development of the Intoxication after Feeding with Fresh Sheep Spleen.

Mouse No.	Coagulation time.				Remarks.
	Before feeding.	After feeding.			
		30 min.	1 hr.	2 hrs.	3 hrs.	
187	5 min., 10 sec.	5 min., 0 sec.	5 min., 20 sec.	7 min., 10 sec.	9 min., 20 sec.	Died.
188	6 " 10 "	6 " 30 "	6 " 20 "	8 " 0 "	8 " 10 "	Ill.

TABLE II.

Experiment II. Amount of Fresh Spleen (Human) Necessary To Produce Delayed Coagulation.

Mouse No.	Amount eaten.	Coagulation time.		Remarks.
		Before feeding.	After feeding.	
	mg.			
317	100	5 min., 10 sec.	8 min., 10 sec.	Ill.
318	100	4 " 0 "	8 " 20 "	Died.
319	50	6 " 0 "	10 " 10 "	"
320	25	4 " 20 "	6 " 20 "	Ill.
321	10	4 " 40 "	6 " 10 "	"
322	10	5 " 0 "	6 " 20 "	Well.
323	5	6 " 10 "	6 " 0 "	"

TABLE III.

Experiments III to VII. Effect of Feeding Spleen of Other Animals to Splenectomized Mice.

Organ fed.	Mouse No.	Coagulation time.												
		First day feeding.						Second day feeding.						
		Before.		After		Remarks.		Before.		After.		Remarks.		
Experiment III.	158	4 min.,	30 sec.	4 min.,	20 sec.			4 min.,	20 sec.	7 min.,	10 sec.	Very ill.		
Spleen (cat).	159	4 "	20 "	4 "	10 "			4 "	40 "	6 "	20 "	Ill.		
	160	5 "	20 "	5 "	30 "			5 "	10 "	5 "	10 "			
	161	5 "	30 "	5 "	40 "			5 "	20 "	8 "	30 "	Died.		
Experiment IV.	119	6 "	10 "	6 "	10 "			6 "	0 "	6 "	10 "			
Spleen (beef).	120	5 "	30 "	7 "	30 "	Ill		7 "	20 "	11 "	30 "	"		
	121	4 "	20 "	4 "	30 "			4 "	20 "	10 "	10 "	"		
	122	4 "	30 "	4 "	20 "			4 "	40 "	6 "	30 "	Ill.		
Experiment V.	178	6 "	0 "	6 "	10 "			6 "	30 "	6 "	30 "			
Spleen	179	4 "	10 "	7 "	20 "	"		7 "	0 "	8 "	10 "	Very ill.		
(guinea pig).	180	4 "	20 "	4 "	30 "			4 "	20 "	4 "	10 "			
	181	5 "	10 "	5 "	20 "			5 "	0 "	5 "	20 "			
Experiment VI.	174	4 "	10 "	4 "	20 "			4 "	30 "	6 "	10 "			
Spleen	175	4 "	20 "	4 "	20 "			4 "	10 "	7 "	40 "	Ill.		
(rabbit).	176	5 "	10 "	5 "	0 "			5 "	30 "	7 "	10 "	"		
	177	4 "	30 "	4 "	25 "			5 "	0 "	6 "	30 "			
Experiment VII.	170	5 "	30 "	7 "	10 "	"		6 "	10 "	7 "	20 "			
Spleen (rat).	171	6 "	0 "	6 "	20 "			6 "	10 "	6 "	20 "			
	172	6 "	10 "	6 "	30 "			5 "	40 "	6 "	0 "			
	173	5 "	20 "	8 "	30 "	Very ill		8 "	10 "	8 "	40 "	Did not eat. Died.		

TABLE IV.

Experiments VIII to XI. Effect of Feeding Gastro-Intestinal Mucosa to Splenectomized Mice.

Organ fed.	Mouse No.	Coagulation time.					
		First day feeding.			Second day feeding.		
		Before.	After.	Remarks.	Before.	After.	Remarks.
Experiment VIII.	34	4 min., 30 sec.	4 min., 20 sec.		4 min., 20 sec.	7 min., 10 sec.	
Stomach	35	5 " 0 "	6 " 10 "		5 " 10 "	7 " 20 "	
(mouse).	36	4 " 10 "	10 " 20 "		4 " 30 "	10 " 30 "	
	37	6 " 0 "	6 " 10 "		6 " 0 "	9 " 20 "	
Experiment IX.	30	6 " 20 "	7 " 0 "		5 " 20 "	9 " 0 "	
Small intestine	31	4 " 40 "	6 " 5 "		5 " 0 "	8 " 20 "	Died.
(mouse).	32	6 " 10 "	6 " 20 "		5 " 30 "	6 " 40 "	
	33	5 " 5 "	5 " 20 "		5 " 5 "	7 " 30 "	
Experiment X.	154	5 " 40 "	7 " 10 "	Ill	6 " 0 "	9 " 10 "	Ill.
Stomach (cat).	155	6 " 20 "	6 " 10 "		6 " 10 "	7 " 20 "	
	156	6 " 10 "	6 " 30 "		6 " 20 "	6 " 20 "	
	157	5 " 10 "	5 " 10 "		5 " 20 "	10 " 40 "	Died.
Experiment XI.	150	4 " 20 "	6 " 10 "		5 " 0 "	8 " 20 "	Very ill.
Small intestine	151	6 " 0 "	6 " 10 "		6 " 10 "	6 " 20 "	
(cat).	152	5 " 30 "	5 " 20 "		5 " 20 "	11 " 10 "	Died.
	153	5 " 20 "	5 " 30 "		5 " 20 "	7 " 30 "	Ill.

TABLE V.

Experiments XII and XIII (a). Effect of Feeding Dried Whole Blood and Bone Marrow to Splenectomized Mice.

Substance fed.	Mouse No.	Coagulation time.					
		First day feeding.			Second day feeding.		
		Before.	After.	Remarks.	Before.	After.	Remarks.
Experiment XII.	182	5 min., 30 sec.	5 min., 20 sec.		5 min., 30 sec.	6 min., 0 sec.	
Dried whole	183	4 " 10 "	7 " 10 "	Ill	7 " 0 "	7 " 40 "	Ill.
blood (sheep).	184	6 " 0 "	6 " 20 "		6 " 10 "	6 " 10 "	
	185	5 " 20 "	6 " 0 "		5 " 0 "	6 " 40 "	"
Experiment	327	4 " 10 "	6 " 20 "				
XIII (a).	328	4 " 30 "	5 " 20 "				
Bone marrow	329	5 " 0 "	7 " 0 "	"			
(man).	340	4 " 20 "	6 " 10 "				

TABLE VI.

Experiments XIII (b) to XX. Effect of Feeding Various Organs to Splenectomized Mice.

Organ fed.	Mouse No.	Coagulation time.				Remarks.
		First day feeding.		Second day feeding.		
		Before.	After.	Before.	After.	
Experiment XIII(b). Lymph nodes (sheep).	83	6 min., 40 sec.	6 min., 20 sec.	6 min., 30 sec.	6 min., 20 sec.	
	84	5 " 30 "	5 " 30 "	5 " 30 "	6 " 0 "	
	85	4 " 20 "	4 " 30 "	5 " 10 "	5 " 30 "	
	86	5 " 10 "	5 " 20 "	5 " 10 "	5 " 10 "	
Experiment XIV. Thymus (sheep).	87	6 " 10 "	6 " 0 "	6 " 0 "	6 " 30 "	
	88	5 " 30 "	5 " 20 "	5 " 10 "	5 " 20 "	
	89	6 " 0 "	6 " 10 "	6 " 20 "	6 " 10 "	
	90	5 " 0 "	5 " 20 "	5 " 10 "	5 " 30 "	
Experiment XV. Large intestine (mouse).	162	6 " 20 "	6 " 40 "	6 " 30 "	6 " 30 "	
	163	6 " 10 "	6 " 10 "	6 " 20 "	6 " 10 "	
	164	5 " 30 "	5 " 20 "	5 " 20 "	5 " 30 "	
	165	5 " 40 "	5 " 30 "	5 " 30 "	5 " 30 "	
Experiment XVI. Liver (sheep).	108	5 " 20 "	5 " 30 "	5 " 10 "	5 " 30 "	
	109	4 " 20 "	5 " 10 "	5 " 10 "	5 " 10 "	
	110	5 " 10 "	5 " 20 "	5 " 20 "	5 " 10 "	
	111	5 " 30 "	5 " 30 "	6 " 0 "	6 " 20 "	
Experiment XVII. Pancreas (sheep).	75	5 " 20 "	5 " 10 "	5 " 30 "	5 " 30 "	Note slight decrease in coagulation time. Compare with salivary gland (Experiment XXV).
	76	4 " 10 "	4 " 30 "	5 " 20 "	4 " 0 "	
	77	5 " 20 "	5 " 20 "	6 " 20 "	4 " 10 "	
	78	5 " 10 "	4 " 40 "	6 " 10 "	4 " 20 "	
Experiment XVIII. Pituitary body (sheep).	100	6 " 20 "	6 " 10 "	6 " 20 "	6 " 20 "	
	101	6 " 10 "	6 " 10 "	6 " 30 "	6 " 10 "	
	102	5 " 10 "	5 " 0 "	5 " 20 "	5 " 30 "	
	103	6 " 15 "	6 " 10 "	6 " 0 "	6 " 0 "	
Experiment XIX. Brain (sheep).	104	6 " 0 "	6 " 10 "	5 " 20 "	5 " 30 "	
	105	6 " 10 "	6 " 0 "	6 " 0 "	6 " 0 "	
	106	5 " 20 "	5 " 10 "	4 " 40 "	5 " 0 "	
	107	6 " 20 "	6 " 30 "	6 " 10 "	6 " 30 "	
Experiment XX. Kidney (mouse).	112	6 " 0 "	6 " 10 "	6 " 10 "	6 " 30 "	
	113	6 " 0 "	6 " 30 "	5 " 30 "	5 " 20 "	
	114	5 " 20 "	5 " 10 "	5 " 10 "	6 " 0 "	
	115	4 " 30 "	4 " 40 "	4 " 40 "	5 " 0 "	

TABLE VII.

Experiments XXI to XXVI. Effect of Feeding Various Organs to Splenectomized Mice.

Organ fed.	Mouse No.	Coagulation time. First day feeding. Before.	First day feeding. After.	First day feeding. Remarks.	Second day feeding. Before.	Second day feeding. After.	Second day feeding. Remarks.
Experiment XXI. Lung tissue (mouse).	127	6 min., 10 sec.	6 min., 0 sec.		6 min., 0 sec.	6 min., 0 sec.	
	128	5 " 0 "	5 " 20 "		5 " 10 "	5 " 20 "	
	129	6 " 10 "	6 " 10 "		6 " 10 "	6 " 20 "	
	130	6 " 20 "	6 " 10 "		6 " 30 "	6 " 30 "	
Experiment XXII. Muscle (sheep).	123	5 " 0 "	5 " 10 "		5 " 15 "	5 " 10 "	
	124	4 " 30 "	4 " 20 "		4 " 20 "	4 " 20 "	
	125	5 " 10 "	5 " 30 "		5 " 20 "	5 " 10 "	
	126	6 " 0 "	6 " 10 "		6 " 10 "	6 " 10 "	
Experiment XXIII. Thyroid (sheep).	79	4 " 50 "	4 " 30 "		4 " 30 "	4 " 40 "	
	80	5 " 10 "	5 " 0 "		5 " 20 "	5 " 30 "	
	81	5 " 10 "	5 " 20 "		5 " 10 "	5 " 30 "	
	82	6 " 30 "	6 " 10 "		6 " 10 "	6 " 10 "	
Experiment XXIV. Adrenals (man).	115	6 " 10 "	4 " 10 "	Ill } Second reading at 6 hrs.	5 " 10 "	5 " 30 "	Ill. } Second reading at 6 hrs.
	116	5 " 30 "	5 " 30 "	"	5 " 20 "	4 " 10 "	
	117	5 " 30 "	3 " 10 "	"	5 " 40 "	6 " 10 "	
	118	5 " 20 "	4 " 0 "	"	5 " 0 "	5 " 10 "	"
Experiment XXV. Salivary gland (mouse).	313	5 " 10 "	2 " 30 "	Died, 18 hrs.			Second reading at 2 hrs.
	314	6 " 20 "	3 " 10 "	" 10 "			
	315			Did not eat	5 " 30 "	2 " 10 "	Died, 4 hrs.
	316			" " "	5 " 40 "	3 " 30 "	" 2½ "
Experiment XXVI. Testicle (rabbit).	330	5 " 20 "	5 " 30 "		5 " 30 "	5 " 30 "	
	331	5 " 10 "	5 " 0 "		5 " 0 "	5 " 20 "	
	332	6 " 10 "	6 " 30 "		6 " 0 "	6 " 0 "	
	333	4 " 30 "	5 " 0 "		5 " 0 "	5 " 0 "	

TABLE VIII.

Experiments XXVII to XXX. Control Experiments Relating to Figures Previously Presented. Intact Mice Fed with Various Organs.

Organ fed.	Mouse No.	Coagulation time. First day feeding. Before.	After.	Remarks.	Second day feeding. Before.	After.	Remarks.
Experiment XXVII. Spleen (sheep).	42	5 min., 20 sec.	5 min., 10 sec.	Amount eaten 0.0 gm.	5 min., 5 sec.	5 min., 0 sec.	Amount eaten 0.340 gm.
	43	4 " 10 "	4 " 40 "	" " 0.065 "	4 " 40 "	5 " 10 "	" " 0.185 "
	44	5 " 30 "	5 " 40 "	" " 0.185 "	5 " 10 "	5 " 10 "	" " 0.08 "
	45	5 " 40 "	5 " 10 "	" " 0.08 "	6 " 5 "	5 " 20 "	" " 0.225 "
	46	5 " 30 "	6 " 0 "	" " 0.06 "	6 " 10 "	6 " 20 "	" " 0.068 "
	47	6 " 0 "	6 " 5 "	" " 0.095 "	6 " 20 "	6 " 10 "	" " 0.275 "
Experiment XXVIII. Small intestine (mouse).	45	5 " 5 "	5 " 0 "		5 " 20 "	5 " 10 "	
	46	4 " 20 "	4 " 30 "		5 " 0 "	5 " 10 "	
	47	5 " 0 "	5 " 10 "		5 " 30 "	5 " 40 "	
Experiment XXIX. Stomach (mouse).	42	5 " 30 "	5 " 5 "		5 " 10 "	5 " 30 "	
	43	5 " 30 "	6 " 0 "		5 " 20 "	5 " 30 "	
	44	6 " 30 "	6 " 20 "		6 " 10 "	6 " 0 "	
Experiment XXX. Salivary gland (mouse).	328	6 " 0 "	4 " 30 "	Ill } Readings at			
	329	5 " 30 "	3 " 0 "	Died } 2 hrs.			
	330	6 " 30 "	5 " 10 "	"			

Other experiments were made to determine the effect of feeding the regular diet of oats and bread on the coagulation time of the blood. Neither the intact nor the splenectomized animals showed any change after eating this food.

The intoxication as described bears a certain general resemblance to peptone poisoning. The fact that it is developed from the intestinal tract likewise suggested that the effects might be due to imperfectly detoxicated digestion products. In following up these suggestions we have studied the reaction of mice to Witte's peptone. We have been unable to poison mice by feeding this substance. When intraperitoneal injections of this peptone are given, an intoxication is produced which causes a delay in the coagulation of the blood and which may lead to death. The change in coagulation time develops more slowly. It is not apparent in six hours but is well developed at eighteen hours. No difference could be detected between the reaction of intact and splenectomized mice to Witte's peptone.

If those splenectomized mice, which, having been fed with either spleen or gastro-intestinal mucosa, survive, are fed continuously with the same substance, a certain number of them will die at later feedings. The remainder after ten days or two weeks no longer develop the evidence of illness, even though they may eat large quantities of material. They do not become sick and the coagulation time of the blood remains normal.

Many important questions raised by the demonstration of this tolerance have not as yet been clearly answered in our experiments. We have much reason for considering that this is an acquired tolerance or immunity produced by the repeated feedings in the susceptible animal. Our figures show certain instances in which animals definitely ill with a definitely delayed coagulation time fail to show reactions at subsequent feedings. These instances are, however, small in number and up to the present time few of our observations have been made with this point sufficiently in view. On the other hand, we know that mice immediately after splenectomy differ considerably in their susceptibility to the intoxication. After a number of weeks they become less susceptible spontaneously. In just what degree the natural process operates and in how far we

influence it by repeated feedings we are unable to decide at present. When we speak of this tolerance as acquired we express our present belief but recognize it as possible that the repeated feedings may have served chiefly to select the resistant animals.

We also have certain evidence that this tolerance is in considerable degree specific in the sense that the animals tolerant to spleen following repeated feedings with this organ are still susceptible to intoxication with gastro-intestinal mucosa and *vice versa.* We hope to extend our observations in this direction in the near future.

In most of the experiments tabulated above the result has been decisive. Certain instances when this has not been entirely the case require comment.

1. Thyroid feeding gives no change in the coagulation time of the blood. The animals may, however, become ill and some may die with an acute intoxication.

2. Suprarenal gland when fed to mice gives rise in most instances to an acute or subacute intoxication with some peculiar characteristics. The coagulation time of the blood is not slowed. It may possibly in some instances be hastened.

3. Administration of pancreas seems to shorten the coagulation time of the blood appreciably. In no instance have we been able to see evidence of illness.

4. The salivary gland of the mouse when fed to mice causes a violent fatal intoxication. The mice do not behave exactly as in the case of the spleen-fed mice and the coagulation time of the blood is also shortened.

These intoxications with thyroid, suprarenal, and salivary gland are important in this connection, as they might be confusing to one who was not aware of the possibility of their occurrence. We feel justified in putting them to one side as having no connection with the subject at hand for the reasons, first, that splenectomized mice are no more susceptible than normal mice in either instance; secondly, that the animals which die do not show the hemorrhagic lesion so frequently found in animals dying after eating spleen or gastro-intestinal mucosa; and, finally, because the coagulation time of the blood either is not altered or is diminished. The intoxications with thyroid and suprarenal glands bear some resemblance to

the picture of such a poisoning which could be drawn on the basis of the well known properties of these organs. That with salivary gland was entirely unforeseen and seems to offer an entirely new subject for consideration.

The experiments presented in which an intoxication of definite character is shown to result from feeding splenectomized mice with either fresh spleen or the mucous membrane of the stomach and upper small intestine are of course difficult if not impossible of interpretation at present.[2] The reaction being so closely limited to these organs, together with the fact that no direct distinction can be made between the intoxication developed in either case, makes it seem reasonable to suppose that there is some close interaction between the spleen and the upper gastro-intestinal mucosa with respect to some function or functions. It is interesting that such an interrelationship has been put forward in the interpretation of experiments of a very different nature.

Luciani[3] reviews the observations of a number of workers in this connection. It is stated to have been shown that an extract of the spleen taken at the height of digestion is capable of activating the zymogens extracted from the pancreas. *In vivo* experiments are said to have rendered it probable that this activation is not only possible but is an important factor in proteolytic digestion. A similar form of interactivity has been supposed to exist between spleen and stomach, but has not been demonstrated experimentally.

Except for the meager suggestion which may be offered by these physiological writings we know of nothing in the literature which is in any way related to the facts as we have observed them. Until more observations are at hand a hypothetical discussion of the subject would be without profit.

SUMMARY.

The intoxication which is developed when splenectomized mice are fed with fresh spleen is more regular in occurrence when the feeding experiment is carried out four or five days after splenectomy than when it is done at later periods. The intoxication is easily recognizable even in its less severe forms by a lengthening of the coagulation time of the blood.

[2] The experiments with dried blood and with bone marrow should in the future receive equal consideration with those in which spleen is used. Up to the present, extraneous circumstances have prevented our giving much attention to the reaction to these substances.

[3] Luciani, L., Physiologie des Menschen, Jena, 1906, ii, 80, 101.

An intoxication of the same character is produced when splenectomized mice are fed with the mucous membrane of the stomach and upper small intestine. Bone marrow and dried blood probably give the same reaction in a somewhat milder form. The other organs either give no intoxication at all when fed, or in certain instances the thyroid, adrenal, and salivary gland (mouse) give intoxications of a different character which affect intact mice and splenectomized animals equally.

The spleen or the gastro-intestinal mucosa is equally effective in producing the intoxication, whether it be derived from heterologous or homologous species.

Certain experiments, not reported in detail, indicate that the susceptibility to the intoxication disappears in time and that this time may be shortened by repeated feedings with sublethal amounts of organ substance.

THE FUNCTION OF THE SPLEEN IN THE EXPERIMENTAL INFECTION OF ALBINO MICE WITH BACILLUS TUBERCULOSIS.

Third Paper.

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(Received for publication, June 23, 1915.)

In our second paper under this title[1] we were able to state that the increased resistance which splenectomized mice show to infection with the tubercle bacillus is decreased again if the infected animals are fed continuously with fresh spleen. The natural conclusion would follow from this that the feeding of the spleen had served to restore some function of this organ which makes for the normal susceptibility to the infection. The situation was, however, extremely complicated by the fact that splenectomized, uninfected mice were found to suffer from a more or less severe intoxication when fed with fresh spleen. The administration of any poison to mice infected with the tubercle bacillus, even in doses which are considerably below those necessary to cause symptoms in the normal animal, is apt to shorten life. We were at that time of writing, therefore, unable to reach a satisfactory conclusion in regard to the issues involved.

Since then we have made a more extensive study of the intoxication to which splenectomized mice are subject when fed fresh spleen.[2] As a result of this study we attained command of a method which seemed to make it possible to determine whether or not feeding of fresh spleen would restore a function to the body which affects its susceptibility to infection in definite degree.

[1] Lewis, P. A., and Margot, A. G., *Jour. Exper. Med.*, 1915, xxi, 84.

[2] Lewis and Margot, *ibid.*, 1915, xxii, 347.

The facts on which we rely for the purpose of the present paper are the following. When recently splenectomized mice are fed with fresh spleen or with the fresh mucous membrane of the stomach and upper small intestine an acute intoxication is produced. In its immediate manifestations the intoxication is the same, whichever of these organs is administered. A number of weeks after splenectomy the mice are less susceptible to this intoxication and in time they can no longer be affected. This return to the normal condition of tolerance can be hastened by the continuous feeding of the fresh organ in sublethal doses, beginning a few days after splenectomy. In this way we have been able to produce groups of mice which between two and three weeks after splenectomy can be fed fresh spleen and fresh gastro-intestinal mucosa with entire impunity.

It is unimportant for the purposes of the present discussion to decide upon the nature of this tolerance: whether, for example, it is an acquired immunity or a selection of naturally resistant specimens. It is, on the other hand, absolutely essential to a proper interpretation of the results, that the tolerance exist at the time stated, two or three weeks after splenectomy, for the following reason.

We have previously stated that the resistance given against infection with the tubercle bacillus gradually disappears, being entirely gone in six months. We present figures in this paper (Experiment II, Group k) to show that in as short a time as ten weeks the specific effects of splenectomy have disappeared, or at least have been reduced to an insignificant residuum. It is obvious that experiments which depend for their interpretation on the presence or absence of this resistance must be carried out in such a way that the animals are infected from two to three weeks after splenectomy, at a time when the resistance is at a maximum and when, as we have repeatedly shown, it is almost uniformly present. On the basis of these considerations we have carried out the following experiment.

A number of mice were splenectomized and separated into three main groups. The first group (Table I, Experiment I, b) was retained on the regular diet. The second group was fed fresh spleen continuously in addition to the regular diet, beginning on the fourth

day after splenectomy and throughout the course of the experiment. A considerable number of this group died, following the first few feedings, of the acute intoxication we have considered. Those surviving in the third week appear in Table I (Experiment I) as Groups c, d, e, and f, according to the source of the spleen with which they were fed. The third group was fed in exactly the same way with stomach or small intestine, and the survivors appear in Table I (Experiment I) as Groups g and h. The animals of all the groups were now, in the third week, inoculated intraperitoneally with 1 mg. of a culture of the tubercle bacillus, Bovine C. A group of intact, normal mice was also inoculated in the same way, as controls. The feedings were continued during the course of the infection. The results of the experiment are shown in Table I (Experiment I).

TABLE I.

Experiment I. Intraperitoneal Infection with 1 Mg. of Culture Bovine C.

Group.	Mice.	Treatment.	Days lived.
a	Intact	None	19, 21, 29, 29, 32, 34, 39, 42.
b	Splenectomized	"	3, 9, 42, 44, 61, 87, 89.
c	"	Fed beef spleen	29, 38, 52.
d	"	" sheep "	26, 28, 30, 36, 37, 61.
e	"	" rabbit "	24, 28, 40, 41.
f	"	" human "	29, 30, 34, 37, 39.
g	"	" stomach (mouse)	9, 57, 71, 94.
h	"	" small intestine (mouse)	2, 78, 79, 90, 126, one still living.

The results of this experiment seem clear. The normal mice (Group a) were all dead by the forty-second day. Disregarding, as we are accustomed to do in this work, the animals dying in less than ten days, the splenectomized mice which received only the normal diet (Group b) lived much longer than this (forty-two to eighty-nine days). The results with these groups repeat our fundamental experiment showing the increased resistance following splenectomy, and furnish the standards for comparison with the following groups.

The mice fed with fresh spleen continuously, with two exceptions were dead in the same time as the normal controls. The resistance given by splenectomy is therefore abolished by feeding spleen. The two exceptions cannot be held of serious account. The experiment

contains so many possibilities for failure that it is rather surprising that a larger number of exceptional cases do not occur. The most obvious possibility in this connection is that these exceptional mice may not have eaten sufficiently or with sufficient regularity to produce the result attained with the larger number.

The animals fed with the gastro-intestinal mucosa lived as long as or longer than the unfed, splenectomized mice. This result seems to dispose entirely of the objection which might be offered on the basis of the spleen feeding alone that the shortening of life could be the result of an added low grade intoxication.

The experiment as a whole is convincing evidence that as a specific result of feeding fresh spleen the resistance to tuberculous infection is lowered to the normal level again.

Because of its ability to produce in splenectomized mice an intoxication with the same general character and intensity as that produced by the administration of spleen, the feeding of the gastro-intestinal mucosa forms the most convincing evidence that the effects produced are in fact the restoration of a true splenic function as related to the specific infection. This being clear, another experiment which we had carried out at an earlier date may be reported, as it is now susceptible of interpretation. In this case, reported here as Experiment II (Table II), other important organs were fed, in comparison with the spleen and certain spleen products.

TABLE II.

Experiment II. Intraperitoneal Infection with 1 Mg. of Culture Bovine C.

Group.	Mice.	Treatment.	Days lived.
a	Intact	None	10, 15, 17, 20, 21, 22, 22, 24.
b	Splenectomized	"	40, 48, 48, 50, 58, 89.
c	"	Fed sheep spleen	14, 19, 22, 26, 26, 29.
d	"	" watery extract of sheep spleen	13, 29, 32, 36, 36.
e	"	" residue after water extraction	28, 30, 32, 34, 36, 87.
f	"	" sheep liver	1, 1, 58, 58, 68, 80.
g	"	" " thymus gland	1, 3, 48, 58, 73, 84.
h	"	" " thyroid "	5, 13,* 17,* 29,* 47, 50.
i	"	" " lymph nodes	1, 3, 51, 54, 58, 59.
j	"	" " pancreas	5, 40, 48, 64, 82, 90.
k	"	None	10, 19, 21, 24, 24, 25, 28, 30
	10 weeks before		

* Died immediately after feeding.

This experiment differs from the first one in that no effort was made to create a tolerance to spleen before the infection was established. The result shows that while the tolerance is a necessary feature in eliminating an important objection to our interpretation of this sort of experiment, it is not at all essential to the success of the experiment itself.

Table II shows that the loss of resistance as a consequence of spleen feeding is due to the restoration of a function probably peculiar to that organ. The resistance is not affected by feeding liver, thymus gland, lymph nodes, pancreas, or thyroid gland. On the same point our first experiment (Table I) shows that it is not affected by feeding gastro-intestinal mucosa. In our earlier papers it was shown that when fresh muscle was fed the resistance was likewise unaffected. It would be desirable from this point of view to feed certain other organs or tissues, notably the bone marrow.

The results of Experiment I and those reported in our second paper[3] show that the source of the spleen is a matter of indifference; whether it is derived from mouse, sheep, rabbit, beef, or human being, the result is essentially the same.

DISCUSSION.

We have reached a point in our consideration of the relation of the spleen to the tuberculous infection in the mouse where it seems possible and advantageous to discuss the subject in more abstract terms. Until it is shown by experiment to be otherwise, we shall in the future attribute the specific properties of the spleen in its relation to tuberculosis to the activity of a single substance. For convenience we may call this substance tuberculosplenatin, a name suggesting merely its origin and its apparent relationship to tuberculosis.

Tuberculosplenatin we consider to be a substance peculiar to the spleen in the same way that adrenalin is peculiar to the suprarenal gland. It is found in the spleen of several different mammals. Its action can be demonstrated by the use of mice as we have described, either by removing the organ and following the course of the infec-

[3] Lewis and Margot, *ibid.*, 1915, xxi, 84.

tion, or by following the course of the infection in splenectomized mice to which the substance is restored by feeding. While tuberculosplenatin exists in other mammals than the mouse we have not so far been able to demonstrate its activity when these other mammals are infected, presumably because of the presence of other factors which obscure its action.

Since the feeding experiments bring results, we must conclude that tuberculosplenatin is able to resist the activity of the gastric juices to a certain extent at least. We have made a beginning in the study of its physical and chemical properties. The results reported in Experiment II above with Groups d and e indicate that the substance is soluble in cold water but that it is difficult to extract completely in this way.

Tuberculosplenatin acts by producing an increased grade of susceptibility or by diminishing the resistance of the animal to infection. There is very little if any evidence as to the mechanism of the action. Murphy and Ellis[4] believe that the increase in resistance following splenectomy is a consequence of an increase in the circulating lymphocytes that follows the operation. If this is true, the substance we are considering might possibly act in restraining the freedom of action of these cells. We have made some blood counts to control this point in connection with Experiment I. We find, as did Murphy and Ellis, that there is a definite lymphocytosis following splenectomy. This has persisted, however, up to the time of infection in spite of the feeding of spleen, and while our observations are not extensive enough to be final we cannot at present attribute the loss of resistance in our experiments to a depression of the number of circulating lymphocytes. The enlargement of the lymph nodes as a result of splenectomy has not been sufficiently marked in our experience to be significant. It has certainly been no less marked in those animals receiving spleen in the food than in the others. For the present, in view of the almost unlimited possibilities for experimental observation in connection with this line of work, we are disposed to regard attempts to explain the reactions we have encountered as of secondary interest.

[4] Murphy, Jas. B., and Ellis, A. W. M., *Jour. Exper. Med.*, 1914, xx, 397.

SUMMARY.

Experiments are reported which show that in all probability the increased resistance to tuberculous infection which is imparted to mice by the removal of the spleen is a consequence of the loss of a function of the organ. This function can be restored by the feeding of fresh spleen. For the present we attribute these changes to the removal and restoration, as the case may be, of a particular substance for which the designation tuberculosplenatin is suggested. This substance is assumed to be related to the spleen as adrenalin is related to the adrenal gland. It is peculiar to the organ but not to the species. It is not found in other organs of the body so far as our observations have extended. The absence of the substance from the lymphatic glands seems of especial importance in this connection.

THE NUMERICAL LAWS GOVERNING THE RATE OF EXCRETION OF UREA AND CHLORIDES IN MAN.

II. The Influence of Pathological Conditions and of Drugs on Excretion.

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(Received for publication, April 17, 1915.)

INTRODUCTION.

In a previous communication (1) we have considered the laws governing the rate of excretion of urea and chlorides and their variations in individuals with normal excretion. The present paper deals with the application of these laws to the study of pathological conditions, and to the study of the action of certain drugs on excretion. The aim is for the present not primarily to draw conclusions regarding the diseased conditions themselves, but to offer a means of obtaining more accurate information regarding these conditions than has hitherto been possible. Certain cases which illustrate the nature of the observations made possible by the method have been selected for presentation.

The laws formulated by Ambard for the excretion of urea are already in clinical use in France (2). In the clinic the method has been used almost solely as a measure of renal function, the variations in the values obtained with Ambard's coefficient serving as an indication of disturbed function. Widal, Ambard, and Weill (3) have studied the excretion of chlorides in edematous subjects, and have made some observations on the effect of diuretics. Bauer and Habetin (4) and more recently Bauer and von Nyiri (5), working in Ortner's clinic in Vienna, have published observations, obtained by means of Ambard's laws, on urea and chloride excretion in a considerable number of cases. Inasmuch as they have used an altogether unreliable method for the determination

of urea in the blood, and publish figures as high as 0.779 gm. of urea per liter of serum in individuals with normal excretion, a figure which a person with normal excretion could attain only after ingestion of enormous quantities of urea, our figures can in no way be comparable with theirs.

With the more accurate and rapid methods for urea and chloride determinations now available, requiring only a small amount of blood, it is possible to make frequent and repeated simultaneous observations on urea and chloride excretion in relation to the concentration in the blood in the same individual, and at a minimum of discomfort to him. A wider field is thus opened for investigation. Quantitative studies of human bodily functions could heretofore be carried out only to a limited extent. With the methods here presented, we have a direct measurement of one of the most important excretory functions, elimination of urea through the kidneys, and also a standard for judging the concentration of chlorides in the plasma in its relation to normality. That these methods are more delicate than other methods used for study of disturbed renal function is apparent from the data presented.

Methods of Study.

Simultaneous observations on urea and chloride excretion are made in patients in a manner identical with that described in the preceding paper (1). One-half hour after the patient drinks 150 to 200 cc. of fluid, the bladder is emptied and the subject takes no further fluid or food until a carefully timed period, usually of seventy-two minutes, is ended. The urine excreted during this period is collected, and at the middle of the period about 10 cc. of blood are withdrawn from an arm vein, clotting being prevented by a small amount of powdered potassium oxalate. The choice of a period of seventy-two minutes is merely for the sake of convenience, seventy-two minutes being one-twentieth of twenty-four hours. A one or two hour period may, of course, be used, all calculations in any case being made on a basis of twenty-four hours. In case an error of a few minutes is made in the time of collection of the second specimen, the calculation should be made on the basis of the time actually elapsed between the voiding of the first and second specimens. The amount of urea in the whole blood, and total chlorides, estimated as sodium chloride, in the oxalated plasma after centrifugalization, are determined. Both urea and chlorides are determined in the urine. By substituting the values obtained in the proper formulas the relationship of the rate of excretion of these substances to their concentration in the blood is determined. In the case of urea the rate of excretion under the conditions found is directly measured in terms of the normal, by the use of the following formula:

$$\text{Index of urea excretion} = \frac{8.96 \times \text{Gm. urea per 24 hrs.} \times \sqrt{\text{Gm. urea per liter of urine}}}{\text{Wt. in kilos} \times (\text{Gm. urea per liter of blood})^2}$$

In the normal individual this formula gives a value of about 100 for the index. The theoretical concentration of sodium chloride in the plasma, under the conditions found, is calculated from the following formula:

$$\text{Calculated plasma NaCl} = 5.62 + \sqrt{\frac{\text{Gm. NaCl per 24 hrs.} \times \text{Gm. NaCl per liter of urine}}{4.23 \times \text{Wt. in kilos}}}$$

For the derivation and significance of these formulas the reader is referred to the foregoing paper. Analyses are carried out by the methods there indicated, and calculations are made with the slide-rule there described.

Observations are made as frequently as desirable. Since they involve no discomfort to the patient, other than the slight inconvenience incident to taking blood, daily observations are usually made when changes in the state of the patient are taking place. The results may be obtained in full within an hour after collection of the specimens, so that information is furnished more rapidly than in other ways. As the observations involve neither additions to the diet nor introduction of foreign substances, they interfere in no way with the dietetic régime or with the progress of the case. The calculations are independent of nitrogen and chloride intake. Gastro-intestinal disturbances, such as vomiting and diarrhea, therefore, do not affect the results, and it is unnecessary to keep the patient on an analyzed diet. Observations can be made at any desired time in almost every case.

Catheterization has been performed only when necessary or advisable in order to avoid danger of losing the specimen, as, for instance, during delirium. It should be practiced in cases in which there is residual urine. Water is given before the seventy-two minute period in order to avoid conditions simulating retention due to dehydration. In the great majority of cases a rate of flow of urine for the period corresponding to 1,000 cc. or more per twenty-four hours is obtained. In practice we make it a rule not to accept results on urea excretion calculated from a urine flow at a rate of less than 500 cc. in twenty-four hours, since results obtained under the condition of a very low rate of urine excretion are apt to be misleading. The chloride formula appears to hold even for very low rates of urinary output.

Pathological Conditions Affecting Excretion.

Conditions Associated with Retention.—Retention of nitrogen and salt have long been recognized as occurring in certain forms of cardiac and renal disease. By the use of the present methods new light is thrown on the mechanism of this retention. The salt retention of pneumonia, for example, has been shown to be entirely different in mechanism from the salt retention of chronic nephritis. For our purpose we propose to consider retention only in the sense of its relation to excretion. That is to say, our conception of retention, in the sense just defined, is a relatively high concentration

of substances in the blood in order to induce sufficient excretion. That retention in the tissues themselves occurs is often lost sight of in studies of renal function by methods which require a comparison of intake and output of certain substances. It is manifestly wrong to consider the kidneys responsible for the failure to excrete a certain amount of salt given by mouth, if the salt is taken up by the tissues and the concentration in the plasma remains low. But if the concentration of urea or chlorides in the blood or plasma remains proportionately high, and the rate of excretion proportionately low, it is correct to speak of retention in the sense of a failure to excrete properly. This is the condition in certain types of cardiorenal, or renal disease.

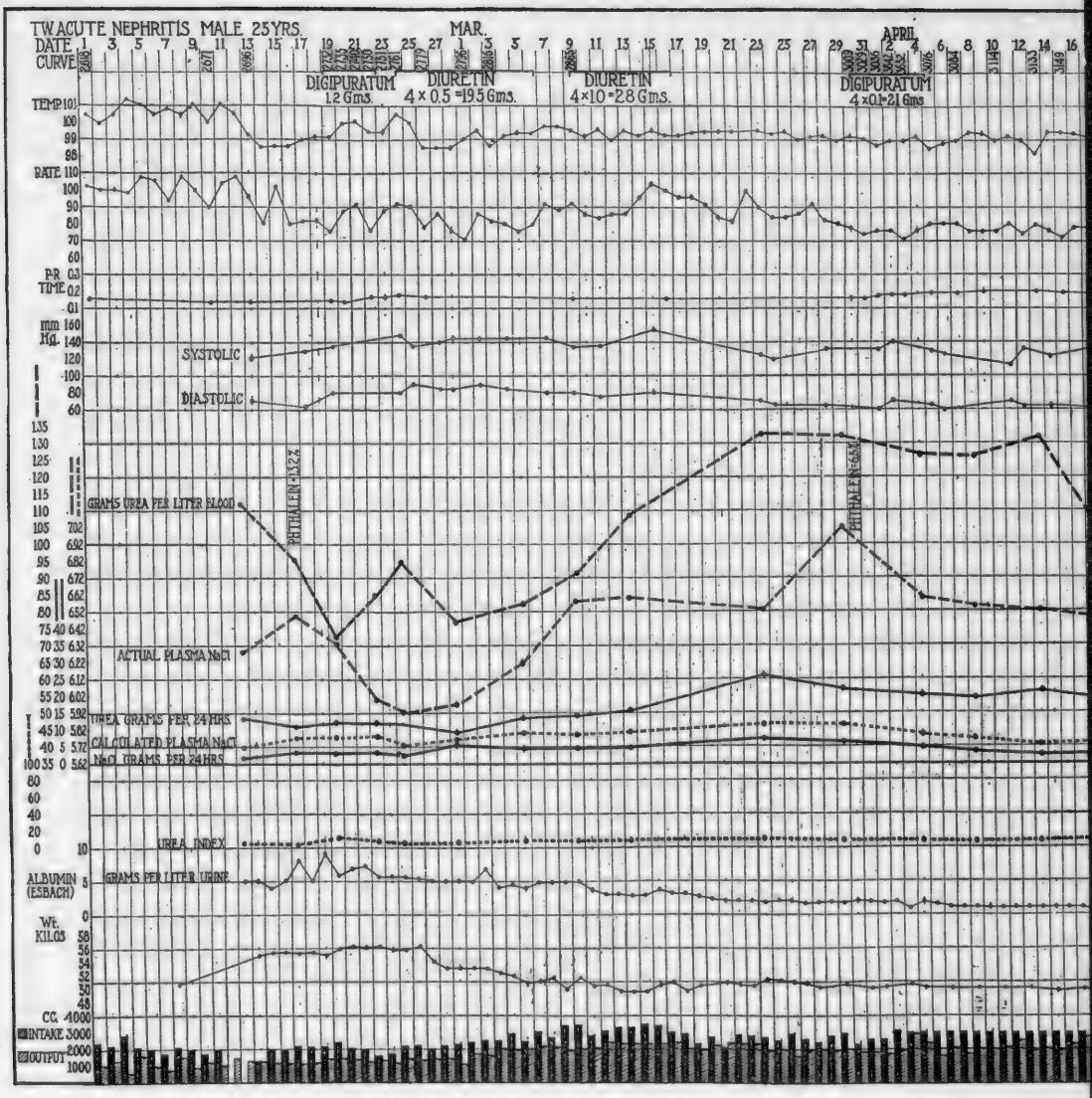
Retention, thus defined, is not an accumulation, but a higher level of substances in the blood, to compensate for increased difficulty in passing through the outlet. It does not mean that the kidney is not able to excrete the normal amount of urea, for the daily excretion, as has repeatedly been shown, may be quite normal. As much may, in fact, be excreted as is absorbed, and the individual be in perfect nitrogen balance (6), although the blood urea or blood nitrogen is much increased. But it does mean that an increased amount in the blood is required in order that increased pressure may be provided to cause the same rate of excretion through diseased kidneys that would be carried on through normal kidneys with a smaller amount in the blood. Actual accumulation may and does occur, under certain conditions. It differs from retention in the above sense, because there is a failure to excrete substances as rapidly as they are absorbed or formed. The picture is different from the purely compensatory phenomenon just described.

The abnormal individual who has retention comes to an equilibrium at a level which is different from that of the normal individual. By comparing his rate of excretion with the standard normal, under the conditions of concentration found in the blood and urine, we obtain a measure of the degree of compensation required to secure the necessary rate of excretion. The necessity for increased pressure may be due to diminished outlet, because a certain amount of the kidney tissue is absent or is not functionating. Thus a per-

son with only one normal kidney, the other being removed, should have an index of urea excretion of approximately 50, 100 representing normality. On the other hand, the kidney cells present may be functionally deficient, though still able to carry out their functions if the pressure under which they work is increased. In either case the index measures the relative functional capacity for the excretion of urea. An index of 100, or 100 per cent, is the standard normal, based on a considerable number of observations on normal individuals. An index of 25 indicates that the individual under observation is excreting urea at 25 per cent of the normal rate under the conditions of concentration of urea found in the blood and urine. An index of 80 is considered as the lower limit of normality, and no normal individual should give a lower figure, providing the fluid intake is sufficient to prevent dehydration.

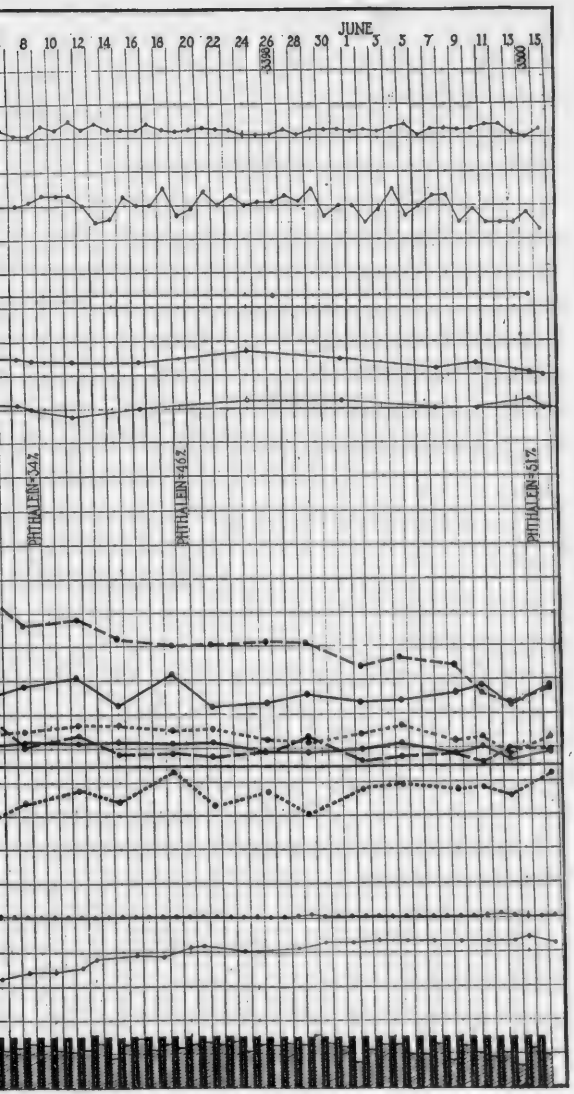
Observations.

Case I (Text-fig. 1) provided opportunity for making repeated observations during the course of acute nephritis, occurring as a sequel of pneumonia and empyema. Besides fluid intake and output, weight, concentration of albumin in the urine, systolic and diastolic blood pressure, P-R time measured electrocardiographically, pulse rate, and temperature, Text-fig. 1 illustrates the findings as regards urea and chloride excretion, with occasional observations on the rate of excretion of phenolsulphonephthalein. The concentration of urea in the blood and the rate of excretion are shown, together with the index of urea excretion, calculated as above indicated. It will be seen that during the first two months of the disease, though the level of the blood urea fluctuated considerably, due to changes in the nitrogen intake, the index remained remarkably constant at 6 to 8. The first fall in blood urea followed an initial restriction of diet, and was accompanied by a proportionate decrease in the rate of excretion. As the nitrogen intake increased, the blood urea rose steadily, reaching a level of 1.3 grams per liter and maintaining this level for some time. The rate of excretion at the same time increased proportionately, so that the index remained constant. When functional improvement did occur it was accompanied by a rapid



TEXT-FIG. 1. Case I. Acute nephritis, postpneumo

XT-FIG. 1. Case I. Acute nephritis, postpneumonia. Partial recovery.



(McLean: Laws Governing Rate of Excretion in Man.)

fall in the blood urea, and an increase in the index toward normal. The rate of excretion during and following this fall remained practically the same as when the high concentration of urea in the blood was found. The maintenance of a constant relationship of rate of excretion to the concentration in the blood, at 6 to 8 per cent of normal, under fluctuating conditions of nitrogen intake, indicates that the process of excretion was being carried out under laws just as definite as those governing the normal excretion. During this period the actual rate of excretion maintained was as high as later in the disease when the conditions were more nearly normal. During recovery the only changes were the fall in concentration of urea in the blood and the rise in the value of the index. One must conclude, therefore, that the high concentration of urea in the blood was maintained in order to compensate for the difficulty in passing through a damaged outlet in the form of kidneys which were functionating at a rate only 6 to 8 per cent of normal. When functional conditions improved, it was no longer necessary to maintain the concentration of urea in the blood at so high a level, and it promptly fell to within normal limits. The relation of the rate of phthalein elimination to the urea index is shown in this case. Both increased at the same time, the increase being parallel. The failure of the index to reach the normal figure for some time, in spite of the fact that the blood urea was within normal limits, shows that the blood urea figure alone was not sufficient in this case to indicate the functional disturbance which still existed.

Text-fig. 1 also shows the rate of chloride excretion, maintained at a low level by a salt-poor diet; the theoretical concentration of sodium chloride in the plasma, calculated from the rate of excretion from the formula given above; and the concentration of sodium chloride found in the plasma by analysis. A marked discrepancy is seen between the actual and calculated concentrations of sodium chloride in the plasma, the former being always higher, and reaching at one time a level of over 7 grams per liter. A return of the plasma chlorides toward the theoretical concentration was much slower than the fall in blood urea, and confirms previous experience that chloride function may be disturbed for some time after ap-

parent recovery. The case also shows the independence of the urea and chloride functions, since the urea function improved so rapidly some time before any change in the chloride relations was visible.

Drug experiments in this case were entirely negative. Edema was present at the start, but disappeared rapidly under free catharsis and sweat baths. The course of the edema is indicated by the weight chart. The later increase in weight was a healthy increase and was not accompanied by any edema.

Case II (Table I) is one of acute nephritis following pneumonia, similar to, but much milder than, the previous one. The urea index remained fairly constant at about 75 for a time, and then rose to a point considerably higher than normal. The chloride excretion followed a similar course, the concentration of sodium chloride found in the plasma being higher than the theoretical concentration during the first stage, and lower during the stage in which the urea index was high. This represents a stage of vascular irritability, which has been described qualitatively by Schlayer (7) and others, but this method offers a means of measuring such changes quantitatively. Recovery in this case is accompanied by a return of both urea and chloride functions toward normal.

TABLE I.

Case II.

Date (1915).	Weight.	Urine per 24 hrs.	Urea.				Sodium chloride.					Urine findings.		
			Gm. per liter of blood Ur.	Gm. per liter of urine C.	Gm. per 24 hrs. D.	Index of excretion I.	Gm. per liter of urine C.	Gm. per 24 hrs. D.	Gm. per liter of plasma.			Blood.	Albumin.	Casts.
									Calculated.	Actual.	Difference.			
	kilos	*cc.*												
Mar. 9	51.0	1,100	0.562	26.2	28.8	83	0.9	0.99	5.69	5.60	−.09	++	H.T.*	+++
" 10	51.0	2,070	0.577	20.9	43.3	104	0.7	1.5	5.70	5.65	−.05			
" 11	51.0	1,195	0.627	27.0	32.3	75	0.6	0.7	5.67	5.81	+.14	0	F.T.	+
" 12	51.0	1,200	0.557	23.3	28.0	77	2.0	2.4	5.75	5.97	+.22			
" 13	51.0	1,600	0.506	16.4	26.3	71	5.2	8.3	5.92	6.15	+.23	0	V.F.T.	+
" 18	51.0	2,034	0.547	15.35	31.2	72	9.3	18.9	6.14	6.17	+.03		V.F.T.	Few.
" 22	53.0	1,500	0.557	17.1	26.6	58	8.3	12.5	6.02	6.08	+.06		V.F.T.	"
" 26	53.8	1,440	0.457	16.3	23.4	75	10.9	15.7	6.10	6.06	−.04		V.F.T.	++
Apr. 2	56.0	6,000	0.321	9.0	54.0	250	7.3	43.8	6.33	6.03	−.30		V.F.T.	++
" 9	56.2	6,300	0.308	10.0	63.0	330	9.8	61.8	6.52	6.21	−.31		V.F.T.	+
May 30	57.0	1,500	0.250	15.9	23.8	240	13.5	20.2	6.17	6.19	+.02		V.F.T.	Few.

*In Table I, H.T. signifies heavy trace; F.T., faint trace; and V.F.T., very faint trace.

Case II.—(Table I.) D. C., male, aged 16. Diagnosis, lobar pneumonia, acute nephritis. Admitted Mar. 8, 1915, on 6th day of pneumonia. Temperature 105.5° F. Consolidation posteriorly in right lower lobe. Blood pressure 112 systolic, 60 diastolic. Mar. 10. Crisis, followed by rapid disappearance of physical signs of consolidation. No edema at any time; no further abnormal physical signs, except for slight pallor. Mar. 26. Discharged from hospital, feeling well except for slight weakness. Blood pressure 102 systolic, 60 diastolic. May 30. Patient has been working for a month; feels perfectly well. Blood pressure 108 systolic, 70 diastolic.

In Case III (Table II) enormous pressure was required in order to induce the necessary rate of excretion of urea and sodium chloride. The close agreement of the urea index with the rate of phthalein excretion is also seen in this case.

Attention has been called by Widal and Javal (8), and more recently by Folin, Denis, and Seymour (9), and by Frothingham and Smillie (10) to the reduction in blood urea or blood nitrogen brought about by giving a low protein diet. They state that in cases with high blood nitrogen, such as in this case (Case III, Table II), no marked reduction occurs on a nitrogen-poor diet. That any considerable reduction is difficult in a case such as this may be shown mathematically. Were this patient to attain a blood urea figure of 0.42 grams per liter, that is to say, one within normal limits, the total urea excretion per twenty-four hours in 1 liter of urine would be 1 gram, the index remaining at 1.2. The formula would then read $\text{Index} = \frac{8.96 \times 1\sqrt{1}}{42.1 \times (0.420)^2} = 1.2$. As the concentration of urea is practically the same in all the tissues as in the blood, before the concentration of over 2 grams per liter could be reduced to 0.42 grams per liter this patient would have to excrete nearly 80 grams of urea already in the body. As the rate of excretion would diminish in proportion to the square of any diminution in the concentration in the blood, it would require an infinite time for the blood urea figure to reach the low level, even if the diet could be kept so that only 1 gram of urea were formed daily. The practical impossibility of reaching such a result is obvious. The comparative ease with which a patient with an index of urea excretion more nearly normal would reach a blood urea figure within normal limits when on a low nitrogen diet may easily be seen.

Case III.—(Table II.) J. O. S., male, aged 13. Diagnosis, chronic nephritis, mitral stenosis. Admitted Jan. 19, 1915, with generalized edema, dyspnea, frequent nausea, and vomiting. Blood pressure 185 systolic, 135 diastolic. Urine clear, straw colored, specific gravity 1008, acid, no sugar. Albumin, 6.5 gm. per liter (Esbach). Many large granular casts, hyalin and epithelial casts, epithelial cells, and leucocytes. No red cells. Eye grounds normal. Jan. 20. Phenolsulphonephthalein, 2 per cent excreted in 2 hours. Jan. 26. Left hospital unimproved. Died one month later.

TABLE II.

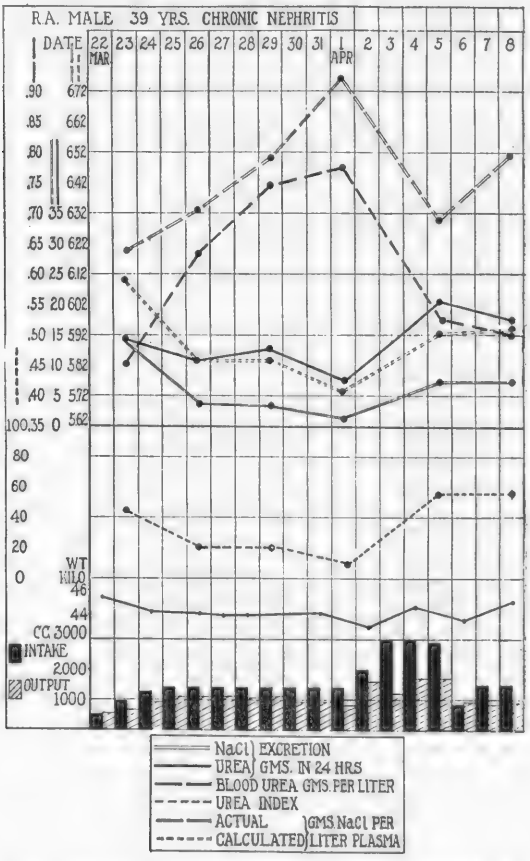
Case III.

Date (1915).	Weight.	Urine per 24 hrs.	Urea.				Sodium chloride.				
			Gm. per liter of blood Ur.	Gm. per liter of urine C.	Gm. per 24 hrs. D.	Index of excretion I.	Gm. per liter of urine C.	Gm. per 24 hrs. D.	Gm. per liter of plasma.		
									Calculated.	Actual.	Difference.
	kilos	*cc.*									
Jan. 20...	42.1	889	2.147	8.8	7.82	1.0	1.5	1.33	5.71	6.55	+.84
" 22...	42.1	960	2.203	9.37	9.0	1.2	2.0	1.92	5.75	6.59	+.84

Case IV (Text-fig. 2) illustrates an actual accumulation of urea and chlorides, due to restriction of fluids while on a full diet. That the power of excreting substances in high concentration in the urine is diminished in certain forms of nephritis is well recognized. It is quite apparent that a fluid output insufficient to carry off the waste products formed will lead to actual accumulation of these products in the body. This condition is manifested, in the case presented, by the increasing urea and chloride content of the blood and the diminishing index of urea excretion. When the fluid intake is increased, urea, chlorides, and index return rapidly to their former state. This case also illustrates the necessity of careful control of the fluid intake in any experiments on nitrogen or chloride balance. That a washing out of urea and chlorides occurs in both normal and pathological individuals with a high fluid output is recognized and expressed in the formulas. Any experiment aiming to determine the balance between nitrogen or chloride intake and output, which does not take the fluid output into account, is apt to be misleading.

Conditions similar to those occasioned by disturbance of function due to renal disease may also be found in heart failure, with passive

congestion of the kidneys. In case the circulation alone is at fault the conditions return to normal when the congestion is relieved by digitalis or otherwise.



TEXT-FIG. 2. Case IV. Chronic nephritis. Blood pressure 185 systolic, 130 diastolic. Urine clear, amber, specific gravity 1023, acid, no sugar, albumin 2 gm. per liter (Esbach). Numerous hyalin, few granular casts. Red blood cells and white blood cells. Mar. 23 to Apr. 2. Fluids restricted, food and salt *ad libitum*. After Apr. 2 fluids increased and food and salt restricted.

Case V (Table III) illustrates the findings in a typical case of heart failure with passive congestion of the kidneys, which was

restored to a functionally normal state by digitalis. During the condition of passive congestion, as evidenced by cyanosis, edema, and albuminuria, the blood urea was high, the urea index low, and the plasma chlorides high in relation to the rate of excretion. Under the action of digitalis the blood urea fell, the index rose to normal, and the plasma chlorides fell until the concentration agreed with the theoretical concentration as calculated from the rate of excretion. The urine became albumin-free and no casts were to be found, indicating complete relief from the congestion which had caused disturbed function.

Case V.—(Table III.) M. H., male, aged 53. Diagnosis, aortic and mitral insufficiency, cardiac failure, passive congestion of kidneys. Admitted Jan. 2, 1915, with dyspnea, cyanosis, edema of extremities. Blood pressure 143 systolic, 110 diastolic. Urine dark amber, cloudy, specific gravity 1027, acid, albumin +++, numerous hyalin and granular casts. Jan. 8. Condition unchanged by rest in bed; digitalis therapy started (digipuratum 0.1 gm., 4 times daily). Jan. 12. Patient much more comfortable, edema disappearing, fluid output in excess of intake. Jan. 17. Digitalis discontinued; total 2.9 gm. Jan. 19. No edema, or dyspnea. Urine albumin-free, no casts. Blood pressure 120 systolic, 75 diastolic. Patient continued in good condition up to time of discharge on Apr. 11.

TABLE III.

Case V.

Date (1915).	Weight.	Urine per 24 hrs.	Urea.				Sodium chloride.					Urine albumin, gm. per liter (Esbach).	Medication.
			Gm. per liter of blood Ur.	Gm. per liter of urine C.	Gm. per 24 hrs. D.	Index of excretion I.	Gm. per liter of urine C.	Gm. per 24 hrs. D.	Gm. per liter of plasma. Calculated.	Gm. per liter of plasma. Actual.	Gm. per liter of plasma. Difference.		
	kilos	*cc.*											
Jan. 4..	83.1	500	0.567	27.3	13.65	24	3.3	1.65	5.71	5.84	+.13	1.5	
" 8..	77.0	420	0.597	30.8	12.94	25	3.35	1.41	5.70	6.15	+.45	0.5	
" 12..	74.0	3,020	0.351	11.84	35.7	130	8.9	26.9	6.07	6.12	+.05	0.25	Digitalis.
" 15..	73.0	1,190	0.356	20.7	24.7	100	6.9	8.21	5.88	5.91	+.03	0	"
" 27..	71.0	1,620	0.328	15.7	25.5	110	7.7	12.5	5.96	5.85	−.11	0	
Feb. 16..	73.2	1,000	0.286	16.42	16.42	100	12.4	12.4	5.99	5.96	−.03	0	

Case VI (Table IV) is a case of heart failure in which actual accumulation of urea and chlorides in the blood occurred. This is evidenced by the very rapid rise in the concentration of both urea and chlorides in the blood, without any increased rate of excretion. This picture, with its rapidly diminishing urea index, is quite dif-

ferent from that presented by Cases I and III, in which a persistently high blood urea was accompanied by a constant value for the index.

Case VI.—(Table IV.) A. W., female, aged 13. Diagnosis, mitral insufficiency, auricular fibrillation, cardiac failure, death. First admitted Apr. 17, 1913. Last admission, Dec. 3, 1914, with edema, dyspnea, and cyanosis. Urine amber, cloudy, specific gravity 1030, acid, albumin +++, few granular casts. Blood pressure 120. Dec. 12. Phenolsulphonephthalein, 68 per cent excreted in 2 hours. Patient's condition failed to improve materially under digitalis, and about Dec. 28 she became decidedly worse, gradually stuporous, and more cyanotic. Edema rapidly disappeared during last few days, and blood contained a very high proportion of cells and a low percentage of plasma. Jan. 10. Died, after having been unconscious two days. Autopsy revealed enormously enlarged and dilated heart, with lesion of mitral valve. The kidneys were those of passive congestion.

TABLE IV.

Case VI.

Date (1914).	Weight.	Urine per 24 hrs.	Urea.				Sodium chloride.					Urine albumin, gm. per liter (Esbach).
			Gm. per liter of blood Ur.	Gm. per liter of urine C.	Gm. per 24 hrs. D.	Index of excretion I.	Gm. per liter of urine C.	Gm. per 24 hrs. D.	Gm. per liter of plasma. Calculated.	Actual.	Difference.	
	kilos	*cc.*										
Dec. 12 . . .	37.9	680	0.460	23.75	16.15	88	7.0	4.75	5.90	6.15	+ .25	
" 21 . . .	38.8	540	0.432	22.05	11.9	70	3.3	1.8	5.76	5.95	+ .19	3.0
(1915)												
Jan. 5 . . .	37.0	1,850	0.837	11.13	20.6	24	2.4	4.44	5.83	7.24	+1.41	1.5
" 7 . . .	32.0	1,000	1.38	12.25	12.25	6	3.7	3.7	5.85	7.85	+2.00	2.5
" 8 . . .	30.0	500	2.18	17.85	8.92	2	1.1	0.55	5.69	8.44	+2.75	0.5
" 9 . . .			2.70							8.24		
" 10 . . .			3.36									0.1

We may summarize the findings in cases with retention as follows. In individuals with defective elimination of urea or chlorides, an increased concentration of these substances in the blood is required to compensate for the faulty excretion. Under the conditions of increased concentration in the blood, the actual rate of excretion and the balance between intake and output may be quite as good as in a perfectly normal individual. The increased concentration in the blood is not to be regarded as an accumulation unless the concentration in the blood is increasing and is associated with a diminished elimination. In any given case the index of urea ex-

cretion measures, in its relation to normality, the rate of excretion under the conditions found. Increased chloride concentration in the plasma is shown by comparing the amount actually found with the theoretical concentration, calculated from the rate of excretion. The concentration in the blood and the rate of excretion of urea and chlorides depend not only on the nitrogen and chloride intake, but on the fluid intake and output. Deficient elimination due to passive congestion may be restored to normal if the circulatory failure is relieved.

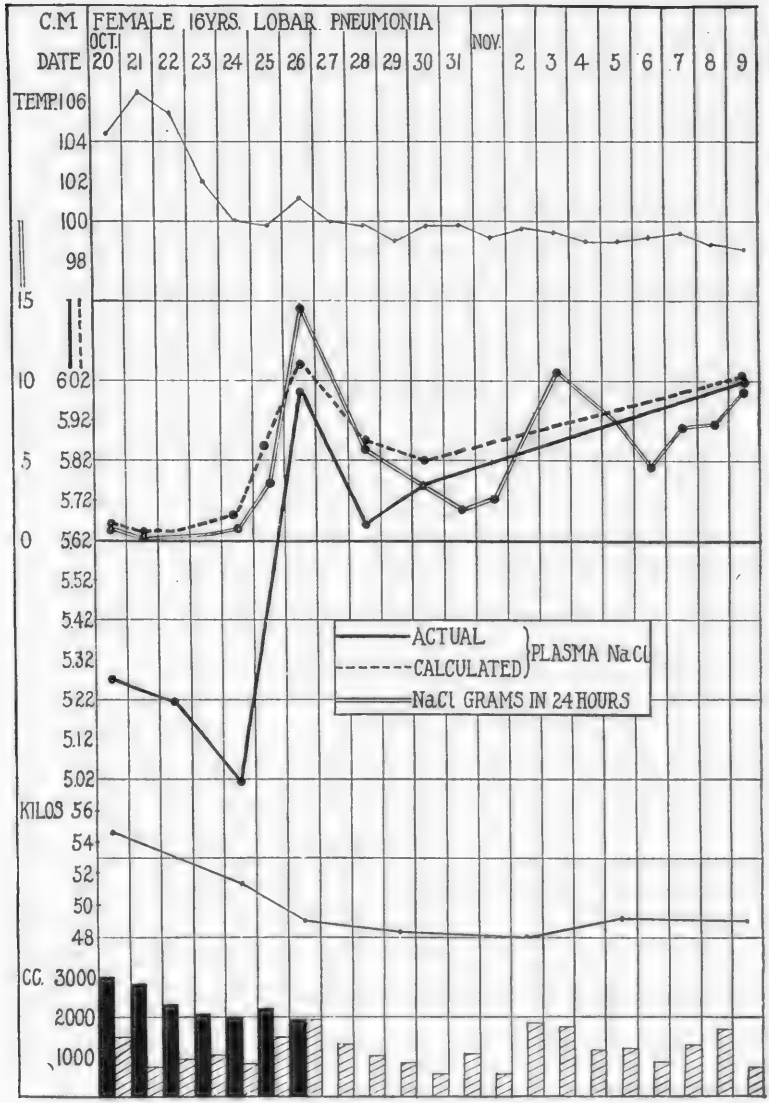
The Chlorides in Pneumonia.

That the chloride retention in pneumonia is associated with a diminished chloride content in the blood has been shown by Peabody (11) and others. Peabody has also shown that the concentration of chlorides in the blood increases at the time excretion begins. This is directly opposed to the condition in nephritis, where chloride retention is usually associated with a high chloride content in the plasma, and suggests at once that the chloride retention occurring in pneumonia and other fevers is not due to a failure on the part of the kidneys to excrete chlorides, as some writers still hold (12).

We have examined the chloride content of the plasma and its relation to the rate of chloride excretion in 60 observations in 13 cases of lobar pneumonia, and have found that the failure to excrete chloride during the acute stage of the disease is almost always associated with a concentration of sodium chloride in the plasma below 5.62 grams per liter (the normal threshold).[1] One must believe that this is the cause of failure to excrete chlorides, since excretion begins with a rise in the chloride content of the plasma.

Case VII (Text-fig. 3) illustrates the findings regarding chloride excretion in a typical case of uncomplicated pneumonia. During the acute stage of the disease, when only a very small amount of chlorides appeared in the urine, the plasma content of sodium chloride was considerably below 5.62 grams per liter. At the crisis the plasma chlorides rose abruptly, and excretion began and continued

[1] Similar findings have been reported by J. Snapper (*Deutsch. Arch. f. klin. Med.*, 1913, cxi, 429) in 5 observations during the chloride retention of pneumonia.



TEXT-FIG. 3. Case VII. Uncomplicated lobar pneumonia, with recovery.

in proportion to the concentration in the plasma. That the low rate of excretion of chlorides in pneumonia is not due to chloride starvation has been repeatedly shown (11). That the low chloride content of the plasma is not due to low intake is shown by the rapid rise in Case VII at the time of crisis, without any increase in the chloride intake. While the finding of a low concentration in the plasma shows that the kidneys are not responsible for salt retention in pneumonia, it does not explain the mechanism of the retention, which requires further investigation.

The Chloride Threshold in Fever.

We have calculated the threshold in cases with fever from the formula used in normal individuals (1):

$$\text{Threshold} = \text{Plasma NaCl} - \sqrt{\frac{\text{Gm. NaCl per 24 hrs.} \times \text{Gm. NaCl per liter of urine}}{4.23 \times \text{Wt. in kilos}}}$$

For the most part the threshold, as calculated by this formula, is actually lowered during the chloride retention of pneumonia, the average threshold in our cases being 5.42 as opposed to the normal threshold of 5.62 grams of sodium chloride per liter of plasma. That a similar condition occurs in other fevers is shown by Case VIII (Table V).

Case VIII.—(Table V.) B. G., female, aged 20. Diagnosis, acute rheumatic fever. Admitted Nov. 20, 1914. Nov. 27. Pain and swelling in joints. Temperature 103° F. Urine amber, cloudy, specific gravity 1019, alkaline, faint trace of albumin, no casts, few leucocytes.

TABLE V.

Case VIII.

Date (1914).	Weight.	Urine per 24 hrs.	Sodium chloride.					
			Gm. per liter of urine C.	Gm. per 24 hrs. D.	Gm. per liter of plasma.			Threshold.
					Calculated.	Actual.	Difference.	
	kilos	*cc.*						
Nov. 27.....	60.0	1,285	2.8	5.1	5.80	5.54	−.26	5.36

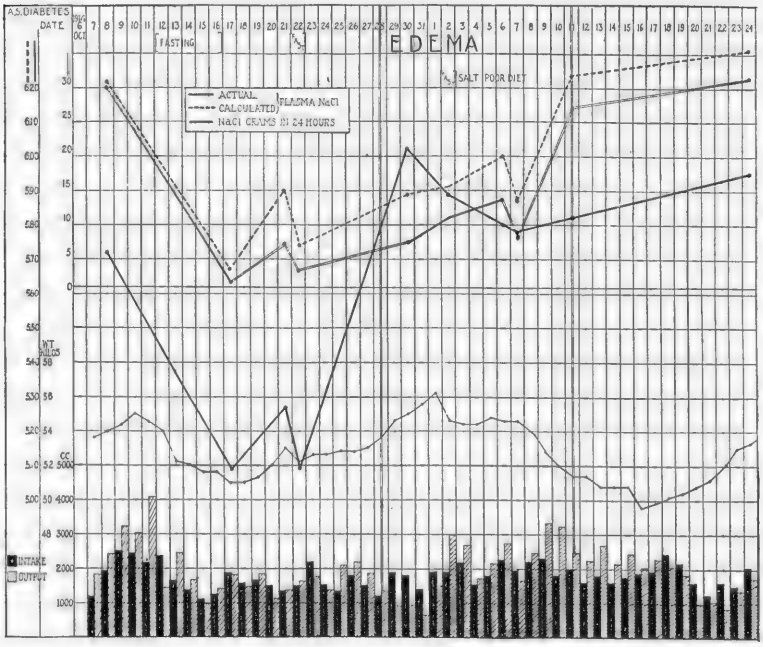
In some instances the threshold was never materially lowered, but in no case was it appreciably raised during the acute stage of the dis-

ease. In one case of chronic interstitial nephritis, dying of pneumonia, with high blood urea, the chloride threshold was considerably lowered. In one case it was raised temporarily during convalescence. As a rule, a lowered threshold returns to normal at the time excretion begins. One case showed a threshold still lowered at the time of discharge from the hospital, after complete recovery.

The findings regarding chlorides in pneumonias and other fevers may be summarized as follows: Chloride retention in pneumonia is associated with a lowered concentration of chlorides in the plasma, and failure of excretion is apparently due to this cause. The threshold is often considerably lowered during fever, and usually returns to normal after the temperature becomes normal. A raised threshold has not been observed during acute fever.

The Chlorides in Diabetes (13).

In 78 observations in 28 cases of diabetes mellitus, mainly severe cases, we have found in a majority a lowering of the chloride threshold. Such a condition is shown in Case IX (Text-fig. 4). In this case, under varying conditions of diet, excretion of sugar, and concentration of sugar in the blood, the plasma chlorides remained proportionately very low, corresponding to a threshold of about 5.12 instead of 5.62 grams of sodium chloride per liter of plasma. Only when the patient developed an edema, such as that to which diabetics are subject, did the plasma chlorides show a relative increase, returning to their previous relationship to the rate of excretion with the disappearance of the edema.



TEXT-FIG. 4. Case IX. Oct. 7. Urine clear, amber; sugar, 75 gm. in twenty-four hours; albumin 0; casts 0. Ferric chloride reaction heavy. Fasted Oct. 12 to 18, with whiskey. No alkalis. Oct. 18. Sugar 0; albumin 0. Ferric chloride reaction very faint. Urine remained sugar-free, with the exception of occasional traces when the increase in diet was not tolerated. Nov. 1. Well marked edema of both feet and ankles, which remained until Nov. 11. Urine, albumin 0, no casts. Had similar edema at subsequent times.

Case X (Table VI) illustrates the findings in a typical case with constantly lowered threshold. In this case the actual concentration of sodium chloride in the plasma is almost invariably below 5.62 grams per liter, the normal threshold.

The average of the threshold calculated by the above formula in 78 observations in diabetics is 5.34, in contradistinction to the average of 5.61 + grams in all normal individuals. Up to the present time we have not succeeded in associating this change in chloride threshold and excretion with any other peculiarities of individual cases of diabetes. It seems to bear no relation to the amount of

sugar excreted nor to acidosis. It seems to occur most commonly in the more severe cases, but has also been seen in milder cases.

Case X.—(Table VI.) J. U., male, aged 44. Diagnosis, diabetes mellitus.

TABLE VI.

Case X.

Date (1914).	Weight.	Urine per 24 hrs.	Sodium chloride.						Sugar in 24 hrs.	Ferric chloride reaction.	Diet per 24 hrs.	
			Gm. per liter of urine C.	Gm. per 24 hrs. D.	Gm. per liter of plasma.						Carbohydrate.	Total calories.
					Calculated.	Actual.	Difference.	Threshold.				
	kilos	*cc.*							*gm.*		*gm.*	
Dec. 3..	45.0	860	1.9	1.63	5.73	5.00	−.73	4.89	2.73	+++	Fasting with alcohol 3d day.	
" 7..	45.0	655	2.4	1.6	5.73	5.08	−.65	4.97	0	+	0	637
(1915)												
Jan. 6..	41.3	1,850	11.13	20.6	6.23	5.35	−.88	4.74	0	+	0	466
May 4..	42.6	950	10.1	9.6	6.03	5.87	−.16	5.46	0	+	40.1	343.5
" 7..	44.0	800	9.8	7.85	5.98	5.22	−.76	4.86	0	+	70	600.5
" 12..	44.0	1,320	9.2	12.1	6.06	5.48	−.58	5.04	Trace	+	10	553
" 13..	43.8	1,590	9.4	14.9	6.12	5.60	−.52	5.10	0	+	20	929
" 18..	44.0	690	10.0	6.9	5.96	5.40	−.50	5.12	Trace	+	15.1	1,526.9
" 22..	44.0	700	14.3	10.0	6.07	5.70	−.37	5.25	0	+	0	1,427
June 2..	43.0	800	9.5	7.6	5.98	5.42	−.56	5.06	0	+	0	1,962

The Influence of Drugs on Excretion of Urea and Chlorides.

Study of the effects of drugs has so far been confined mainly to the action of digitalis. We have found, in the majority of cases examined, a lowering of the chloride threshold as the result of giving digitalis, both in individuals with normal excretion and in cases with passive congestion. The threshold may be brought merely to normal, as in Case V (Table III), or it may be brought temporarily far below normal, as in Case XII (Table VII).

Case XII.—(Table VII.) G. M., male, aged 50. Diagnosis, cardiac hypertrophy, auricular fibrillation. Patient has been under observation since May 19, 1914. Rate remains slow and patient feels well when taking digitalis, but rate becomes rapid and dyspnea and edema appear when digitalis is omitted. Oct. 5, 1914. Patient has had no digitalis since Sept. 27. Pulse rate gradually increasing. Urine clear, amber, specific gravity 1025, neutral, albumin 0.25 gm. per liter (Esbach), no casts found. Oct. 16. Marked dyspnea, face puffy, heart rate rapid. Output of fluid has diminished and weight increased. Digitalis started. Oct. 23. Fluid output increased and patient much better. No edema.

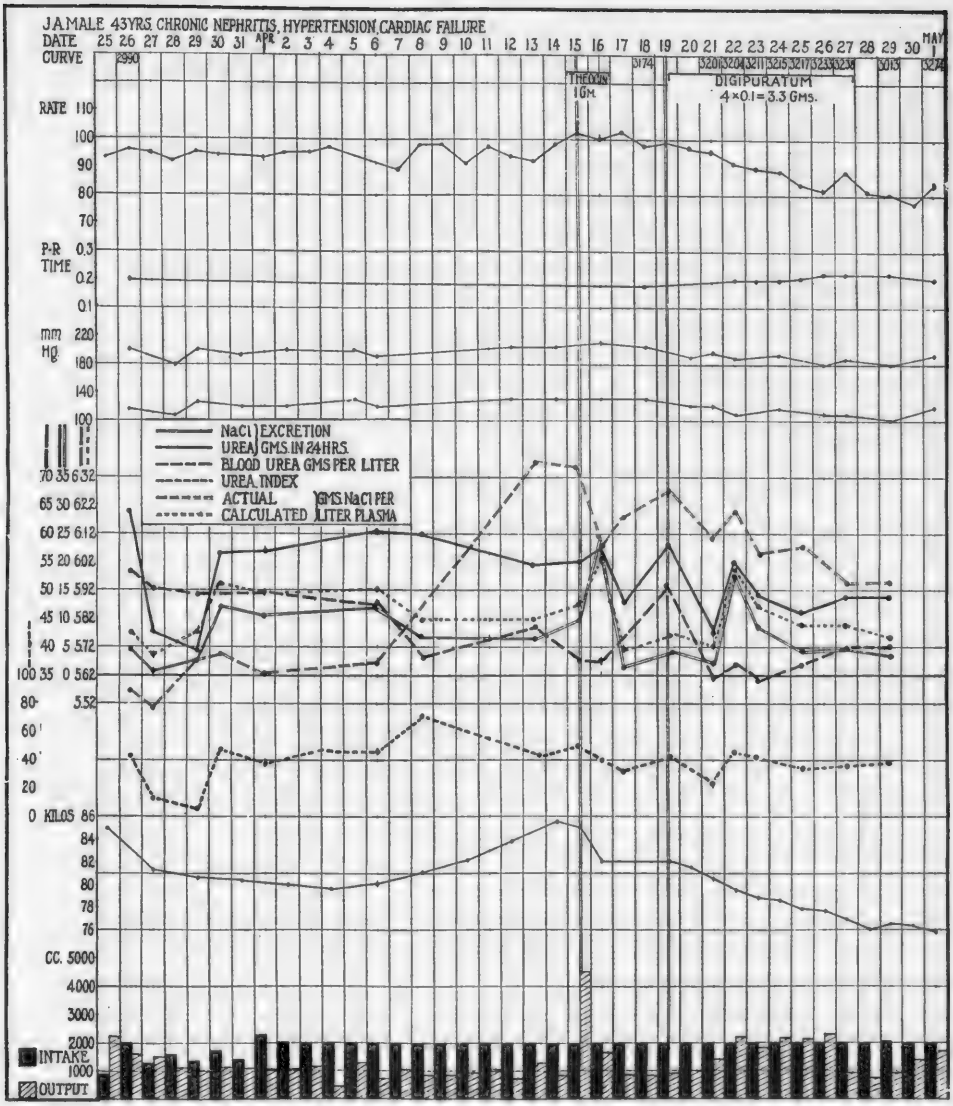
Nov. 2. Patient feels well; rate remains slow. Two similar series of observations have since been made on the same patient.

TABLE VII.

Case XII.

Date (1914).	Weight.	Urine per 24 hrs.	Urea.				Sodium chloride.					Phthalein excreted.		
			Gm. per liter of blood Ur.	Gm. per liter of urine C.	Gm. per 24 hrs. D.	Index of excretion I.	Gm. per liter of urine C.	Gm. per 24 hrs. D.	Gm. per liter of plasma. Calculated.	Gm. per liter of plasma. Actual.	Gm. per liter of plasma. Difference.	1st hr.	2d hr.	Total.
	kilos	*cc.*										*per cent*	*per cent*	*per cent*
Oct. 5..	71.0	760	0.400	18.2	13.85	53	13.0	9.8	5.96	6.11	+.15	38	30	68
" 16..	73.0	685	0.500	26.82	18.37	47	5.1	3.5	5.78	5.97	+.19	Medication.		
" 23..	72.6	1,600	0.352	13.15	21.0	76	10.0	16.0	6.03	5.51	−.52	Digitalis.		
" 26..	72.0	1,680	0.358	13.68	22.9	82	12.3	20.7	6.11	6.11	0	"		
Nov. 2..	72.4	1,580	0.396	14.02	22.3	66	12.1	19.1	6.09	6.10	+.01			

Urea excretion is increased after passive congestion is relieved by digitalis, but digitalis seems to have no specific direct effect on urea excretion. Case XI (Text-fig. 5) shows the effect of both digitalis and theocin, as separate observations on the same individual. Both the chloride and urea findings are illustrated, as in Text-fig. 1. Except for the temporary fall in the urea index at first, caused by insufficient fluid, the index remains practically unchanged, and shows no response to drug therapy. The constantly low index is apparently due to a disturbance in function due to chronic nephritis, with hypertension. When admitted, the patient was under the influence of large doses of infusion of digitalis, and the plasma chlorides were very low, the concentration being below the normal threshold. As the influence of digitalis disappeared, the plasma chlorides rose rapidly, edema appearing and increasing with the increase in plasma chlorides. Diuresis with theocin increased the chloride excretion and brought down the plasma chlorides temporarily to the theoretical concentration. Conditions rapidly returned to the previous state, until digitalis was again administered. At this time the plasma chlorides did not fall to the previous low level, though they responded to digitalis therapy with a decrease in concentration, approaching but not reaching the theoretical concentration.



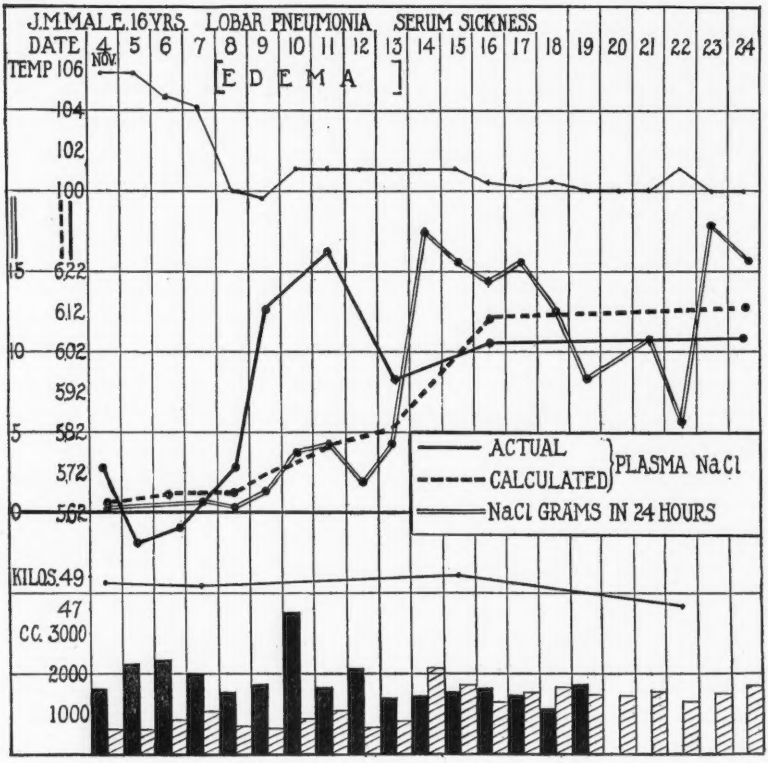
TEXT-FIG. 5. Case XI. Chronic nephritis, hypertension, and cardiac failure. Urine clear, amber, specific gravity 1007, acid, albumin heavy trace, numerous hyalin and granular casts. Admitted under influence of infusion of digitalis. Later treated with theocin and with digipuratum.

Edema, however, disappeared and the patient gave other evidences of a favorable reaction to digitalis. Since this time the patient has been out of the hospital, and has returned with edema. At this later time, not shown in Text-fig. 5, digitalis caused the excretion of 11,000 cc. of urine in forty-eight hours, and the plasma sodium chloride fell to 5.37 grams per liter and remained low for some time. The index of urea excretion was exactly the same during this enormous diuresis as on the preceding day, when the fluid output was only 900 cc. The length of time for which the chloride threshold remains low after digitalis varies in different cases, but it may remain low for ten days or longer after digitalis is discontinued.

Digitalis and diuretin were both without effect in acute nephritis (Case I), so far as could be told by our observations. They were also without effect in Case IV in later observations, not shown in Text-fig. 2. We cannot at present make any general statements regarding the use of diuretics in nephritis, but the method offers opportunity for exact study of the action of various diuretic drugs when applied to diseased conditions.

Relation of Chloride Excretion to Edema.

We are not yet prepared to take up in detail the subject of the relation of chloride excretion to edema, but certain observations here presented refer also to this subject. Cases I, IV, and XI with nephritis; V, VI, and XII with heart failure; and XI with diabetes all showed edema, and all showed a relatively increased concentration of chlorides in the plasma. Case XIII (Text-fig. 6) had a pronounced generalized edema, occurring during serum sickness after pneumonia, after treatment with serum. Though his plasma chlorides had been low during pneumonia, they rose to a high point during the edema, and returned to the theoretical concentration as the edema disappeared. This case is of interest in that it illustrates two types of salt retention occurring in the same patient within a few days. During pneumonia, excretion was low, with a low concentration in the plasma. During serum sickness, excretion was again low, with a high concentration in the plasma. The normal period following the edema serves as a control for the other periods.



TEXT-FIG. 6. Case XIII. Lobar pneumonia; serum-treated. Crisis followed by serum sickness with generalized edema. Nov. 11. Urine, no albumin. Complete recovery occurred.

In Cases V, XII, IX, XI, and XIII disappearance of edema was accompanied by the return of the plasma chlorides to practically the theoretical figure. In Case I edema disappeared long before chloride excretion became normal, though the patient was constantly on a salt-poor diet. Case IV has never been free from edema and has always a relatively high concentration of chlorides in the plasma. Cases with persistent edema and low plasma chlorides have not been seen. Whether the relationship is one of cause and effect, or merely one in which the change in chloride excretion is due to the same cause as the edema, we cannot at present state.

SUMMARY.

A method of measuring the rate of excretion of urea, in terms of the normal, as presented in a preceding paper, has been applied to the study of diseased conditions and of the action of drugs. Simultaneous observations on chloride excretion have been made by comparing the concentration of chlorides actually found in the plasma with the theoretical amount, calculated from the rate of excretion. Observations made in this way have been found applicable to the study of various diseased conditions.

CONCLUSIONS.

1. The rate of excretion of urea in diseased conditions may be measured in terms of the normal by the index of urea excretion, and direct evidence as to the state of one of the more important excretory functions may be thus obtained.

2. The rate of excretion of chlorides gives a basis for calculating the theoretical concentration of chlorides in the plasma. By comparing the concentration actually found with the theoretical concentration, changes in the function of chloride excretion may be studied.

3. Increased concentration of urea in the blood is, as a rule, a compensatory phenomenon, in order to provide sufficient pressure to cause its excretion through a damaged outlet. Under certain conditions actual accumulation occurs.

4. Relatively increased concentration of chlorides in the plasma occurs in certain conditions, especially in certain forms of cardiac and renal disease.

5. Under certain conditions, notably in fevers or in diabetes, or as the action of diuretics (digitalis), the chloride threshold may be temporarily or permanently lowered. This may result in a decrease in the concentration of chlorides in the plasma to a point lower than the lowest point which is seen in normals.

6. Failure to excrete chlorides in pneumonia is associated with a lowered concentration of chlorides in the plasma. Excretion begins at the time this concentration increases.

7. Edema is usually accompanied by a relatively increased con-

centration of chlorides in the plasma. The relations ordinarily return to the normal state when edema disappears.

8. Chloride and urea functions may be quite independent of one another.

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